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THE NEWER KNOWLEDGE OF NUTRITION

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THE NEWER KNOWLEDGE OF NUTRITION

THE USE OF FOODS FOR THE PRESERVATION OF VITALITY AND HEALTH

BY

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To

DR. C. EIJKMAN

Professor of Hygiene
in the University of Utrecht,
Who First Produced Experimentally
a Disease of Dietary Origin,
This Book is Dedicated

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PREFACE TO FOURTH EDITION

Advances along several lines since the publication in 1925 of the third edition of this book make necessary an extensive revision in order to include the recent important studies relating to several phases of nutrition. The general plan of the third edition has been preserved and while much of the former edition has been retained, the book has been essentially rewritten for the purpose of elimination of some parts and the condensation of others so as to make room for the new experimental work without enlargement of the volume.

The bibliography of the third edition contained 1870 references. To cover the entire field of nutrition with the same thoroughness to-day would require the citation of about eight thousand communications. Obviously it was not possible to include a complete bibliography without making the volume unwieldy. Furthermore, the bibliography of nutrition is so easily available in Chemical Abstracts and in the Quarterly Cumulative Index Medicus, which are accessible in numerous libraries, that one would hardly be justified in publishing an exhaustive list of publications on the subject. For these reasons we have included only a select bibliography, but we have made an effort to include all papers which contain important special lists of literary citations.

The original purpose of preparing a comprehensive treatise on the science of nutrition, in which the newer knowledge is presented in its historical setting, has been adhered to. The literature has been examined to May, 1929.

E. V. McCollum.
Nina Simmonds.

Baltimore.

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THE NEWER KNOWLEDGE OF NUTRITION

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CHAPTER I

THE BEGINNING OF A NEW ERA IN NUTRITION STUDIES

Components of Protoplasm.—The first step in reaching an understanding of the chemistry of the living tissues must involve the separation and study of their structural units. This was the field of activity of many organic and biological chemists during the nineteenth century. Through these studies it became established that the body of an animal or the tissues of a plant consist essentially of proteins, carbohydrates, fats, and a series of related substances collectively known as lipins. Besides these, living tissues contain more or less water and a number of inorganic elements. The fats and the simpler substances into which they can be converted in the process of saponification were exhaustively studied. The complex carbohydrates and simpler sugars were discovered, and their chemical relationship made clear. The proteins are the most complex of the food substances and the most difficult to study chemically. Familiar examples of these are egg white, casein of milk, and the principal components of meat. Proteins are rich in nitrogen, the average content being not far from 16 per cent. Certain proteins occurring in plant and animal food-stuffs differ greatly in their physical properties. They are, nevertheless, closely related chemically, since all can be resolved into the same digestion products. Their behavior with the digestive juices produced by the pancreas or alimentary tract and with chemical reagents has been studied with marked success. Certain substances are special products elaborated by certain tissues and under definite conditions. These substances became regarded from the standpoint of nutrition as of subordinate interest. Examples of these are the alkaloids, quinin, strychnin, and cellulose. The latter serves as skeletal tissue for the plant but is not essential for the animal.

In the same category belong the waste products of animal metabolism, such as urea, uric acid, and creatinin, which are not found in plant tissues.

As early as 1840, proteins, fats, carbohydrates, mineral matter, and water were recognized as being never-failing components of plant tissues and were looked upon as the fundamental factors in the diet of animals.

Study of Foods by Chemical Methods.—Investigators in nutrition long labored to discover and to perfect methods for the analysis of foods, in order to determine the amounts of protein, carbohydrate, fat, and ash contained in them. Pronounced differences were observed in the composition of many substances which serve as food for man and animal. Meats, eggs, and a few seeds, such as the pea and bean, are rich in protein. Cereal grains contain much less, and the fruits, tubers and vegetables very little in the fresh condition. Great variations were observed in the water content of foods, and in their yields of fats and carbohydrates. One of the great epochs in the development of the science of nutrition is that in which Voit (1881) in Germany, and Atwater and his associates (1895) in America, examined and tabulated the chemical composition of an extensive list of human foods. Later, agricultural experiment stations accumulated similar data concerning foods used for animals. Previous to 1900 the idea that there was any marked variation in the quality of proteins from different sources was not generally appreciated.

Early Evidences of Deficiency Diseases.—It is remarkable, in the light of the discoveries during the last few years, that students of nutrition accepted for so long without experimental proof the belief that a chemical analysis revealed the dietary values of foods.

Primitive man was sufficiently industrious in most parts of the world to secure for himself a varied diet derived from cereal grains, legume seeds, fruits, roots, tubers, meats, fish, and certain edible leaves. In certain geographical areas he possessed flocks and herds. These provided him with clothing, meat, and milk. Van Helmont called milk "brute's food," and thought it advisable to substitute for it bread boiled in beer and honey. Liebig likewise in his book "Food for Infants" proposed making a substitute for milk.

For centuries in several parts of the world restricted diets have produced diseases in man. It is of interest now to look back

from our present position and point out the problems waiting to be solved.

There had been in the literature of pathology for many years evidences from human experience that a diet may have an appropriate chemical composition and yet be unsatisfactory for the nutrition of man. These observations did not secure from students of nutrition the attention they merited. It had been known for centuries that stale foods produced outbreaks of scurvy among sailors, soldiers and prisoners; it was also known that fresh vegetables and fruits would cure it.

Beri-beri, a peripheral neuritis resulting in paralysis, had been known from remote times. In 1884 it was demonstrated that in the Japanese Navy it was due to faulty diet. Under the direction of Admiral Takaki a ship made a cruise of eight months, during which time the incidence of beri-beri among the sailors was noted. Another ship was then sent over the same course with an improved dietary. The effects were so striking that the diet of the Navy was improved, with the result that the disease practically disappeared. In 1897 Eijkman, a Dutch physician, tested the question of the relation between consumption of polished rice and incidence of beri-beri. He found that pigeons or chickens restricted to a diet of milled rice developed in 2 to 4 weeks a paralysis strikingly suggestive of beri-beri in man. His work showed that there was something in the bran layer which protected against beri-beri. This observation did not influence students of nutrition until Funk called attention to this work in 1911, when he designated the term "vitamine" to indicate a group of indispensable nutritive complexes.

For centuries pellagra was a scourge among the peasants in some parts of Italy and of France; its etiology was thought to be due to the poor quality of the simple diet. This disease was not observed in America until after 1900; after that it increased rapidly up to 1917, especially in the Southern States.

Funk conceived the idea that certain diseases result from faulty diet, the fault resulting in a lack, relative or absolute, of one or another of several substances, each of which when present in the diet protects against a specific type of pathological change. Beri-beri, scurvy, pellagra and rickets were all attributed by him to lack of "vitamines" in the diet. He had experimental evidence of the existence of only one of these specific "deficiency" diseases, viz., beri-beri, or, as it is called when produced experimentally in animals, polyneuritis. Beri-beri was the only disease

which had been studied experimentally in a manner that afforded a basis for judgment as to its etiology.

Funk had no clear conception of the importance of the several factors which constitute an adequate diet. He knew how to produce but one deficiency disease, polyneuritis or beri-beri. This was effected by the method which Eijkman had used in 1897, i.e., restricting pigeons to polished rice. These observations in pathology failed at that time to impress students of animal nutrition. Rice is now known to be deficient in several dietary factors.

The Wisconsin Experiment with Cattle.—In 1906 an experiment was begun at the Wisconsin Experiment Station which had a far-reaching effect on the minds of students of nutrition. It was planned by S. M. Babcock, and was carried out by Hart and Humphrey (1911), with the later coöperation of Steenbock and McCollum. The object of this experiment was to determine whether rations for cattle so made up as to be alike in so far as could be determined by chemical analysis, but derived each from a single plant, would be of equal nutritive value for growth and maintenance of vigor.

The ration employed for one group of animals was derived solely from the wheat plant, and consisted of wheat straw, wheat gluten, and the entire wheat grain. The ration of the second group consisted of the entire corn plant: kernel, stalk, and the leaf,—together with a portion of corn gluten, a by-product of the cornstarch industry. The third group was fed solely from the oat plant: rolled oats, oat straw, and leaf. A fourth group, supposed to serve as controls, was fed a ration having the same chemical composition, but derived from about equal portions of wheat, corn, and oat products.

The animals employed were young heifer calves weighing about 350 pounds; they were comparable in size and vigor. They were restricted absolutely to the experimental ration, plus salt (NaCl). They were allowed to exercise in an open lot free from vegetation. Their behavior during growth and reproduction was extremely interesting. All groups ate practically the same amounts; digestion tests showed that there were no differences in the digestibility of the three rations.

Not until the animals had been confined to the experimental rations for a year or more was differentiation in their appearance easily observable. The corn-fed group was sleek and fine. In marked contrast stood the wheat-fed group. These were rough-coated and gaunt. They were small of girth as compared

FIG. 1.—Photograph of a cow which grew up on a ration derived solely from the corn plant. The seed, straw and leaf of the plant were all included in the food mixture. Her nutrition was excellent, as shown by her appearance, the vigor of her offspring and her ability to produce an abundance of milk. Figure 2 shows a photograph of her calf, taken soon after it was born. Rations consisting of the entire plant may be highly satisfactory. The seed of the plant is never in itself a complete food.

FIG. 2.—Photograph of calf produced by a mother whose ration had been long derived from the corn plant as the sole source of nutriment. It was vigorous and developed normally. The entire wheat plant, seed, leaf and stem make a diet which can support growth, but not good nutrition. The corn kernel alone does not induce growth.

with those fed the corn-plant ration. The weights of the two groups did not differ significantly. The groups fed the oat-plant ration and the mixture of the three plants, leaf and seed, stood intermediate between the lots just described. The animals receiving the mixture of products fared no better than the others. The corn-fed animals were at all times in better nutrition than those receiving the greater variety of materials.

The reproduction records are of special interest. Corn-fed heifers invariably carried their young to full term. The young showed remarkable vigor, and were normal in size, able to stand and suck within an hour after birth, which is the rule with vigorous calves. All lived and developed normally. Young of wheat-fed mothers were the reverse in all respects. They were born 3 to 4 weeks too soon, were small, weighing on an average 46 pounds, whereas the young of corn-fed animals weighed 73-75 pounds each. The latter weight is normal for new-born calves. Young of wheat-fed mothers were either dead when born or died within a few hours. Young of mothers which had grown up on the oat-plant ration were almost as large as those from corn-fed mothers, the average weight being 71 pounds. All cows in this group produced their young about 2 weeks too soon. One of four was born dead, two were very weak and died within a day or two. The fourth was weak but was kept alive. Young of cows fed the mixture of the three-plant products were weak in most cases. One was born dead and one lived but 6 days. The mothers were continued on the experimental rations; the following year they repeated in all essential details the reproduction records observed in the first gestation period.

Records were kept of milk production during the first 30 days of the first lactation period. The average production per day per individual of the corn-fed lot was 24.03 pounds; for the wheat-fed lot 8.04, and for the oat-fed lot 19.38 pounds. The animals fed the mixture of the three plants produced an average of 19.82 pounds per cow per day. In the second lactation period the average figures for milk production were 28.0, 16.1, 30.1, and 21.3 pounds, respectively, per day during the first 30 days.

Through autopsy and analysis of the tissues of the young, analysis of the feeds and excreta, an elaborate attempt was made to find the cause of the marked differentiation of the animals fed the restricted diets. There were marked differences in the character of the fat in the milk of the different lots. The urine of the wheat-fed animals was distinctly acid, whereas that

FIG. 3.—This cow was the same age as that shown in Figure 1. She derived her ration entirely from the wheat plant during the last two-thirds of her growing period. This ration, when submitted to chemical analysis, showed almost exactly the same composition as that fed to the cow in Figure 1. Note the poor nutritive condition of the wheat-fed cow. It has not been possible to prepare a ration solely from wheat products, which will induce good nutrition in animals. Figure 4 shows the typical appearance of the calves produced by cows fed upon a ration properly "balanced," but derived entirely from the wheat plant, and containing the seed, stem and leaf.

FIG. 4.—Calf produced by cow shown in Figure 3. It was born prematurely, weighed but little more than half as much as calves normally do at birth, and was dead when born. The importance of the source of the food supply, both for the nutrition of mother and the unborn young, is strikingly illustrated by these animals. (Figures 1 to 4 are from Research Bulletin 17 of the Wisconsin Experiment Station.)

of the other lots was alkaline or neutral. It was impossible by any means known to chemistry to discover the cause of the differences in the well-being of the cows. Hart, Steenbock, Humphrey and Hulce (1924) reported satisfactory calving in cows on a diet of wheat and wheat straw if this was supplemented with bone-meal, NaCl, and cod liver oil. They believe that the failure to secure good results in the earlier experiments was probably due to calcium and vitamin A deficiency and not to the presence of a toxic substance.

The Purified Food Mixture.—Previous to this experiment, investigators had been unsuccessful in their efforts to nourish young animals on purified protein, starch, sugar, fats, and mineral salts. Lunin (1888) first investigated the significance of inorganic salts in animal nutrition. He found that adult mice could live for several months in good health on a diet of milk, but they invariably died within a month if they received a ration composed of what he believed to be the essential ingredients of milk, i.e., casein, butter fat, milk sugar, and the ash of milk. Commenting upon his experiments he says: "Mice can live quite well under these conditions when receiving suitable foods (e.g., milk), but as the above experiments demonstrate they are unable to live on proteins, fats, carbohydrates, salts and water, it follows that other substances indispensable for nutrition must be present in milk besides caseinogen, fat, lactose and salts." Although Bunge gave this conclusion publicity in his "Physiological and Pathological Chemistry," a book which was widely used at one time, it did not attract the attention it deserved. Others who attacked the problem of the significance of the mineral content of the diet were Steinitz (1898), Leipziger (1900), Zadik (1899), Ehrlich (1900), Socin (1891), Hall (1896), Falta and Nöggerath (1905), Jakob (1906), Marcuse (1896), Henrique and Hansen 1905), and Willcock and Hopkins (1906). In all these trials directed toward the nutrition of animals with food mixtures which contained everything determined in the ordinary food analysis, the animals lost weight from the beginning. They died unless their diet was changed. It was impossible at the time to explain the reason.

Experiments of Pekelharing.—A most remarkable experiment was reported in 1905 by Pekelharing, of the University of Utrecht. It is published in a Dutch journal from which the following quotation is selected.

"When white mice are fed on bread baked with casein, albu-

min, rice-flour, lard and a mixture of all the salts which ought to be found in their food, while they are only given water to drink, the animals starve to death. During the first few days all is well. The bread is eagerly nibbled and the mice look healthy. But soon they get thinner, their appetite diminishes and in four weeks all the animals are dead. If, however, instead of water they are given milk to drink, they keep in good health, though the quantity of albumen, lactose and fat which they assimilate with the milk is quite negligible in comparison with what the bread on which they are fed contains. The element in the milk which keeps the animals alive also occurs in the whey from which casein and fat have been eliminated. Till now my efforts constantly repeated during the last few years, to separate this substance from the whey and get to know more about it, have not led to a satisfactory result, so I shall not say any more about them. My intention is only to point out that there is a still unknown substance in milk, which, even in very small quantities, is of paramount importance to nourishment. If this substance is absent, the organism loses the power properly to assimilate the well-known principal parts of food, the appetite is lost and with apparent abundance the animals die of want. Undoubtedly this substance not only occurs in milk but in all sorts of foodstuffs, both of vegetable and animal origin."

Experiments of Hopkins with Purified Food Substance.—In 1906 Hopkins wrote: "But further, no animal can live upon a mixture of pure protein, fat and carbohydrate, and even when the necessary inorganic material is carefully supplied, the animal still cannot flourish. The animal body is adjusted to live either upon plant tissues or other animals, and these contain countless substances other than the proteins, carbohydrates and fats. Physiological evolution, I believe, has made some of these well nigh as essential as are the basal constituents of diet; lecithin, for instance, has been repeatedly shown to have a marked influence upon nutrition, and this just happens to be something already familiar, and a substance that happens to have been tried. The field is almost unexplored, only it is certain that there are many minor factors in all diets, of which the body takes account. In diseases, such as rickets, and particularly in scurvy, we have had for long years knowledge of a dietetic factor, but though we know how to benefit these conditions empirically, the real errors in the diet are to this day quite obscure. They are, however, certainly of the kind which comprise these minimal qualitative

factors that I am considering. Scurvy and rickets are conditions so severe that they force themselves upon our attention, but many other nutritive errors affect the health of individuals to a degree most important to themselves, and some of them depend upon unsuspected dietetic factors."

In 1912 Hopkins published the results of experiments extending over several years. He reported that when rats were fed a food mixture containing caseinogen, starch, cane sugar, lard and inorganic salts, they remained alive and grew a little when the components of the diet were supplied in a crude condition. When they were carefully purified, growth invariably ceased after a short time and the animals declined and died. He showed that their energy consumption was sufficient so their failure was not due to insufficient food intake. Growth failed when the animals were consuming food in more than sufficient quantity to maintain normal growth.

Hopkins found that a small daily allowance of milk, which in no case supplied more than 4 per cent. of the food eaten, induced a fair amount of growth. These experiments marked the beginning of an appreciation of the importance of what Hopkins has termed the "accessory factors of the diet." It appears that at this time Hopkins was not aware of the epoch-making observations of Eijkman (1897) that a diet of polished rice would induce polyneuritis in birds and that rice polishings contained something which would relieve the condition.

The Rat in Nutrition Studies.—Studies of this character must of necessity be carried out with small animals, because it is laborious and expensive to prepare the purified food-stuffs in sufficient amounts for experiments. It is highly desirable to shorten such experiments as far as possible by the use of short-lived omnivorous animals which grow rapidly and quickly reach maturity. In this way it is possible to accumulate data sufficiently fast to make progress reasonably satisfactory. The domestic rat serves the purpose admirably. It has a gestation period of 21 days and under normal conditions the young are ready to wean at the age of 25 days. A well-nourished female usually produces her first litter at about the age of 90 days; she will have five or more litters by the age of 14 months, which ordinarily marks the end of her period of fertility. The span of life of the well-fed rat is about 36 months. With this animal it is possible within a relatively short time to accumulate data regarding growth and reproduction which would take years with

domestic animals of large size, long period of gestation and long span of life. With the latter the expense would be prohibitive.

Can Inorganic Phosphorus Compounds Meet All Physiological Needs?—One of the earliest explanations occurring to investigators as the possible cause for the failure of the animals was the probable need for phosphorus in certain organic combinations. Some experiments were interpreted to mean that proteins containing phosphorus were better utilized for growth than were phosphorus-free proteins. Between 1895 and 1910 there was a wide-spread belief that the presence of organically bound phosphorus was of great importance. It is easy to appreciate how the idea gained credence. Casein, the principal protein of milk, contains phosphorus, and the yolk of egg is rich in the phosphorized protein, vitellin, and in phosphorized fats. Milk and egg yolk are constituted for the rôle they play in reproduction and the nutrition of young during early post-natal life. It seems significant that in these two substances organically bound phosphorus is abundant, whereas it is not abundant in other foods. Proteins of plants do not contain phosphorus as a part of the molecule. Other organic phosphorus compounds (lecithins, nucleic acids) exist in plant tissues.

A number of experimenters studied between 1898 and 1909 the relative merits of organic and inorganic forms of phosphorus in nutrition. McCollum has reviewed these studies (1909).

Mistaken Conclusion Which Stimulated Research in New Direction.—In 1907 McCollum took up the study of the cause of the failure of animals to grow on mixtures of purified food-stuffs and employed the rat as the experimental animal. He (1909) introduced a new feature into experiments of this type by seeking to increase the variety of food-stuffs in the diet. In this way he hoped to overcome the failure to secure satisfactory nutrition in animals. In the experiments referred to the one condition required of every organic component of the ration was that it should be free from phosphorus. This condition was essentially met. The only source of phosphorus in the diet was finely ground tricalcium phosphate, a natural mineral.

The proteins employed were edestin from hemp seed and zein from maize. Corn starch, wheat starch, milk sugar, glucose, cane sugar, butter fat, bacon fat, and cholesterol, together with the ash constituents, made up the food. Cholesterol is found in most tissues and has physical properties similar to fats. No effort was made to feed a constant mixture. On the contrary,

the diet was changed from day to day as far as possible in order to prevent the anticipated loss of appetite. Several young rats gained weight, one more than doubling its weight in 56 days. This rate of growth would now be considered far below normal. These results were interpreted to mean that growth had been induced by a mixture of purified food substances, where previous attempts had failed. The only difference between the experimental diet used in this study and those of earlier investigators *appeared* to be palatability. Important deductions were drawn from these results concerning synthetic reactions which the body could accomplish.

The above paper is deserving of special notice because it reported the first experiments involving growth with a food supply considered to be essentially a mixture of purified food-stuffs, every one of which could be named. It seemed to demonstrate that the only substances necessary in the diet are proteins, carbohydrates, fats, and certain salts. It appeared to prove that the cause of the failure of the earlier experiments of this type was due to lack of palatability. This was one of the conclusions of the author, since shown to be an error. The data disposed of the disputed question concerning the relative merits of phosphorized and phosphorus-free proteins. They could be interpreted only to mean that the body is capable of synthesizing from salts of inorganic phosphoric acid all of the complex phosphorus-containing organic substances so important as components of living matter.

Since these ideas were accepted as established facts, it seemed that the next great advance in nutrition was the study of the relative value of the numerous proteins of the plant and animal tissues. Chemically they are very differently constituted when derived from different sources. Chemical methods were not entirely satisfactory for the analysis of these complex bodies. Kossel, Fischer, and Abderhalden in Germany, and Osborne in America, found that only a little more than half of the protein molecule could be accounted for in identifiable products of digestion, the amino-acids. Such analyses, although representing an advance in chemical science, were too incomplete to serve as a basis to judge the nutritive values of individual proteins. The way now seemed open for the quantitative comparison of isolated and purified proteins. A single protein could be fed as the sole source of this dietary factor.

Unfortunately for the progress of this branch of physiological

chemistry, the diet with which McCollum had secured growth in young rats was something more than it appeared to be. Five years later it became apparent that the cause of the growth of these rats lay in the chance inclusion in the diet of milk sugar, which was not so pure as it was thought to be, and to the inclusion of butter fat. This diet was not a mixture of proteins, several carbohydrates, fats and inorganic salts, but contained unsuspected nutritive principles. Two of these factors were in the milk sugar, although this was believed, on the basis of careful tests, to be highly purified lactose; the others were uncharacterized substances which the butter fat contained. The failure of all investigators in this field to appreciate these facts led to confusion for several years. The further history of investigations which cleared up this problem will be given in the following chapters.

CHAPTER II

THE EARLIER VIEWS OF NUTRITION PROBLEMS

Beaumont's Views of Nutrition Problems.—Although food constitutes the chief item in the expense of living among the wage-earning class, and is more intimately concerned with the promotion of health and strength than are clothing, shelter and climate, it is only very recently that any serious thought has been given to the nature of food, and to the processes which it undergoes in being utilized for the bodily upkeep. Early man used the food he found available. Latitude, climate, soil, and proximity to large bodies of water determined his dietary habits. Common observation showed that people in different regions of the world lived on diets of widely different character, yet without marked differences in physical development. Indifference to the nature of the food supply, with the exception of palatability, was therefore natural, since there was no obvious evidence that the character of the diet had anything to do with well-being.

The paucity of the knowledge concerning nutrition about the middle of the nineteenth century is illustrated by the views of Beaumont in his book "Physiology and Experiments," published about 1832. He had the good fortune to study digestion with the hunter, Alexis St. Martin, who had a fistulous opening into the stomach as the result of a gunshot wound. Beaumont collected gastric juice from his subject's stomach and studied its effect on various foods. He also introduced foods into the stomach and observed through the opening the behavior of this organ during digestion. He believed the view to be erroneous that there were various kinds of nutrient substances. In his opinion there was but one kind of food, or as he termed it, "aliment," which he believed was present in all foods, and was dissolved out by the action of the gastric secretion. Beaumont was one of the most progressive investigators of his time.

The chemistry of both inorganic and organic substances advanced with great rapidity during the nineteenth century. Among the facts established was that foods contained proteins,

carbohydrates and fats, thus disproving the view that there was but "one kind of aliment." It is the oxidation of these food substances that yields the energy which keeps the body warm, and enables it to do mechanical work. The laws governing energy metabolism were next developed.

Lavoisier Placed Nutrition on Scientific Foundation.—The modern era of the science of nutrition was introduced by the famous French scientist, Lavoisier, in 1780. He was the first to apply the thermometer and the balance to the study of the chemical changes taking place in the living body. He established the fundamental fact that the production of heat was a process of combustion which involved the combination of oxygen of the air with the substances taken as food. He discovered that the temperature of the air had a pronounced influence on the rate at which substances in the body were oxidized. Oxidation is slow at temperatures which we find comfortable with light clothing, and is accelerated by cold. He found exercise to stimulate combustion in the body, and observed that during work it might rise to several times the resting metabolism. These fundamental facts formed the subjects of numerous investigations, which greatly increased our knowledge of detail in this important field.

About 1842 Joule described experiments which established the mechanical equivalent of heat. Heat is measured in terms of the small calorie, which is the amount necessary to raise the temperature of 1 c.c. of water 1 degree C. The calorie used in dietetics is the large or kilo calorie which is the heat required to raise 1 kilo (2.2 pounds) of water 1 degree C. or 1 pound of water approximately 4 degrees F. The rate at which energy metabolism varies with activity, and in the absence of suitable clothing with the temperature, is the most spectacular phase of metabolic processes. A man at rest may need about 1,600 calories a day in the form of suitable food, but at severe labor he may require 10,000 calories a day. It is not surprising that this obviously important phase of metabolism was early studied in detail.

Studies on Energy Metabolism.—Pettenkofer and Voit in Munich (1866) published their studies on energy metabolism. With a respiration calorimeter they measured accurately the amount of oxygen absorbed, the carbon dioxide and the water in the products of respiration, and the heat given off by the body under various conditions. They introduced into their experimental work the idea suggested by Liebig in 1842, viz., that the

nitrogen eliminated in the urine could be made a measure of the protein destroyed in metabolism, since protein is the one prominent food-stuff which contains this element. They studied the metabolism of fasting men and dogs, and of the same subjects when taking different kinds and amounts of food. From the magnitude of the "respiratory quotient," the figure obtained by dividing the volume of carbon dioxide eliminated by the volume of oxygen consumed in respiration $\frac{CO_2}{O_2}$ it was possible to decide what kind of food was being oxidized as a source of energy. When carbohydrate is burned the quotient is 1.0, when fat is burned it is 0.7, and when protein is burned it is approximately 0.8. A dog could be maintained in nitrogen and energy equilibrium during a period of a few days on a diet of protein alone (muscle tissue).

Specific Dynamic Action of Food-stuffs.—Studies of energy metabolism [1] led to the discovery that the protein differs in one marked respect from either carbohydrate or fat, viz., in its stimulating effect upon metabolism as measured by rate of oxidation. This is illustrated by the following type of experiment: If the rate of energy metabolism of a fasting dog is measured, it will be found that it remains constant within narrow limits so long as the animal remains at rest. If now the dog is fed liberally with either fat or carbohydrate, and the observations continued, there is observed a slight increase in metabolism which is the result of the utilization of these foods. If on the other hand, a liberal portion of protein-rich food, such as meat, is fed instead of the non-nitrogenous nutrients, there is an acceleration of metabolic processes, attended with a marked increase in energy set free within the tissues. This was first shown by Bidder and Schmidt in 1852. Their experimental animal was a cat. During a certain interval of time the animal used 50.18 gm. of oxygen and eliminated from its lungs 53.52 gm. of carbon dioxide. It was then allowed to eat meat, and the observations continued. During a similar interval it used 193.84 gm. of oxygen and respired 113.52 gm. of carbon dioxide. This stimulation is due to the specific dynamic action of protein.

The researches of Voit, Pettenkofer, Rubner, Atwater, Lusk,

[1] For a detailed history of this important phase of nutrition, the reader is referred to "The Science of Nutrition," Graham Lusk, 4th ed., Philadelphia, 1928; and to "The Chemistry of Life," L. B. Mendel, New Haven, 1923.

Zuntz, and Benedict on the metabolism of matter and energy in the body attracted deserved attention. For years this kind of study occupied a large area in the field of vision of students of nutrition. The researches were extended to some of the finer problems of metabolism. It was shown that carbohydrate can be converted into fat and that a part of the carbon which the protein molecule contains can be converted in the body into glucose. These investigations shed much light on those conditions of perverted metabolism which prevent the oxidation of sugar (diabetes). In the light of the new developments in the chemistry of proteins and their digestion products, the amino-acids, Lusk explained in great measure the cause of the specific dynamic action of proteins (1915). Lusk (1922) says that in the presence of an abundance of oxidizable fragments of carbohydrates the heat production in metabolism is raised to a high level. The production of increased heat after carbohydrate ingestion may be termed the metabolism of carbohydrate plethora. The same conclusion is reached regarding fat as regarding carbohydrate: That in the presence of a plenitude of fat particles there is a metabolism of fat plethora due to the utilization of fat by special fat receptive cellular affinities. The specific dynamic action of protein consists in a specific chemical stimulus of protoplasm which is independent of the oxidation of the material through which the stimulus is applied. It may be termed the metabolism of amino-acid stimulation.

Chemical Analysis of a Food-stuff.—After 1895 it was demonstrated that proteins differed markedly in their chemical composition. Previous to that time it was widely believed that a chemical analysis showed the nutritive value of a food, and on the basis of such analysis advice was given as to how foods should be combined. The methods in use at that time were first described in 1864 by Henneberg, and were adopted by the German Official Agricultural Chemists. These were afterwards used by food chemists all over the world.

The food analysis consisted of the following determinations: From the nitrogen content the protein was calculated by multiplying by 6.25, on the assumption that all proteins contained approximately 16 per cent. of nitrogen; the fat was estimated by extracting the food with some solvent, usually ether, and weighing the substance extracted from a known amount of food; the "crude fiber," or cellulose, by treating a sample with a solution of acid and then with one of alkali sufficiently strong to

dissolve all components except the more resistant forms of cellulose, and a small amount of mineral matter which this cellulose retained; the ash, or mineral matter, by burning a weighed sample and subsequently weighing the residue left after incineration. The analysis was made on a sample of food which was carefully dried to free it from moisture. The sum of the protein, fat, ash and fiber was subtracted from 100 to give a figure designated "nitrogen-free extract." This was assumed in calculations as essentially a measure of the amount of utilizable carbohydrate contained in the food. This in outline is the chemical analysis of a food. It was shown later that the *biological method* of analysis is essential to complete our knowledge of the nutritive value of foods (page 28).

By 1900 careful determinations had been made of the amount of heat liberated when a definite weight of food is burned; this was known as the calorific or fuel value. Numerous determinations had been made of the amount of energy required per day for a man of a certain weight, when at rest and when doing several kinds of work, representative of moderate and more severe kinds of labor. The illustration most widely used by writers on nutrition was that of comparing the body to an engine, in which fuel (food) was burned and by which mechanical work was performed, with the liberation of a certain amount of waste energy in the form of heat. The adjustment of the supply of calories, or energy units of food, to the needs of the individual, and the amount of protein necessary to make good the "wear and tear" of the tissues, which is an unavoidable result of the life processes, were considered as the two factors of prime importance in the planning of the diet.

Digestion Experiments.—It was appreciated many years ago by students of nutrition that the food analysis had its limitations. The belief prevailed that the way to eliminate the defects of chemical technic was to supplement the data obtained by chemical methods with information secured by trials to determine the digestibility of foods. The subject and the diet having been decided upon, samples of the food to be eaten were carefully analyzed to determine their content of protein, fat, ash, moisture, and "nitrogen-free extract." The period of the experimental trial varied considerably, but in most cases it covered three days. It was customary to keep the subject on a milk diet during a fore period, so that the feces derived from the experimental diet would be different in appearance and could be accurately sep-

arated. The chemical constituents of the ingested food minus the amounts of each which appeared in the fecal residues, afforded the data from which the digestibility of the protein, fat and carbohydrate were calculated.

There were some errors in this procedure. In addition to the food substances which escaped digestion, a part of the nitrogen and salts eliminated from the intestine were derived from the secretions of the digestive glands, and from the cellular elements which came from the intestinal mucosa. The quantity of secretions eliminated from the tract depended in great measure on the content of indigestible cellulose in the food. This acted as a sponge, and retained liquids so that they could not be reabsorbed. An animal fed a diet containing just sufficient protein to meet its needs, may suffer a nitrogen deficit on including in its food a liberal amount of cellulose or other indigestible carbohydrates such as are abundant in many vegetables.

In interpreting the results of digestion experiments "utilization" of the several components of the food was assumed to be measured by the extent to which they disappeared from the digestive tract. That such an interpretation is not always justified is readily understood. A diet of cereal grains will be well digested and absorbed, but the substances which reach the blood and tissues are not a satisfactory pabulum for nutrition. Some complexes which are indispensable are present in too small amounts to admit of the utilization of the other useful ones which are abundant. The latter cannot serve a useful purpose unless they can be used to form definite structures. Such a food supply may be well absorbed but not assimilated. Utilization is an unfortunate choice of a term in this sense for it is liable to be misinterpreted. Knowledge of the digestibility of food-stuffs is of value, but one must not lose sight of the idea of quality in the array of digestion products.

It will be appreciated in the light of what has been said that the chemical analysis of a food, and knowledge concerning its digestibility, even when its palatability is satisfactory, give no information concerning its physiological effects. These effects can be discovered only by observations on the well-being of men or animals restricted *during considerable periods of time* to a definite type of diet. It was assumed for a long time, and without any experimental evidence in support of the assumption, that any diet which complied with the composition of ordinary combinations of foods which experience had shown to be

palatable and wholesome components of the diet, and was so constituted as to furnish the necessary amount of protein and of energy in digestible form, would promote growth and prolong well-being if available in sufficient quantities. This popular conception we now know to be far from true.

Study of Supplementary Foods in Animal Production.— It gradually became the custom in agricultural experiment stations to compare the effects of certain combinations of foods on the growth of young animals, and on milk production in cows. These experiments showed plainly that all rations of similar chemical composition are not alike in their physiological effects. In no instance was it possible, however, because of lack of knowledge of the several factors which enter into the normal diet, and because of the faulty planning and conduct of many of the experiments, to interpret the nature of the causes which determined their outcome. These belonged to the type of *immediate utility* experiment and were fostered by agricultural experiment stations because of their practical nature. They were of great economic value, since they showed clearly that certain combinations of foods were either poor, good or excellent for the nutrition of animals. The experiments were not, because of the nature of the food-stuffs employed, and because of the simple mixtures monotonously fed to the animals, of such a nature as to make possible deductions applicable to problems of human nutrition. Much of this experimental work was done by men with little scientific training. The experiments were therefore poorly planned and controlled, with the result that confusion prevailed.

By 1900 nutrition studies for solving the problems of economic animal production had developed the conception of the specific effects of different foods on the development of the body during growth, and for the performance of work or the production of milk, wool, etc. The skilled animal husbandryman was distinctly in advance of the human physiologist or the medical man in his knowledge of nutrition from the practical standpoint. Atwater, who represented the most advanced thought of his time, wrote in 1895 his book, "Chemistry and Economy of Food." This book clearly reflects his view that if we knew the chemical composition of all the food-stuffs entering into the human diet, their digestibility, energy values, and the energy requirements of an individual at rest and when doing various kinds of work, we should have all the necessary data to enable us to plan a highly

satisfactory nutritive regimen. The well-balanced diet was one in which the proportions among the several recognized nutrients corresponded with the standards adopted as the result of statistical studies of what people in different circumstances and in different localities were selecting for their food when guided by the appetite and cost.

Study of Food Requirements by Statistical Methods.—These dietary studies were all made after the plan introduced by Voit in Germany. Atwater made numerous studies of American dietaries. Much interest was manifested by the public in these results. Others took up similar investigations, so we have available the diets of people in all parts of the country and in all walks of life during the period from 1890 to 1900. The diets of Alabama negroes, Mexican laborers in New Mexico, students in boarding clubs, families of faculty members in several universities, Maine lumbermen, foreign families of various nationalities, as well as workingmen's and professional men's households, were subjected to scientific scrutiny. The quality of any dietary was evaluated by comparison with the standards adopted by Atwater and Voit for energy and protein. This great body of studies were for the most part published in bulletins of the United States Department of Agriculture. Their collection represents an epoch in the development of knowledge of human nutrition.

Mistaken Idea of Economy in Foods.—When knowledge of nutrition had advanced to this point, it was natural that the idea of economy in food should attract a great deal of attention, because on the average half or more of the family income is spent for food. In the household of a wage-earner food is the largest single item in the family budget. Atwater made calculations concerning the amounts of protein, fat, carbohydrates and fuel values which could be purchased for twenty-five cents. These results made it clear that at that time (1894) this sum in Massachusetts would buy one-sixth of a pound of protein, one-fifth of a pound of fat, and 1,120 calories of energy if expended for sirloin steak. The same amount spent for oysters at 50 cents a quart purchased one ounce of protein, two ounces of total nutrients and 230 calories of energy. In buying wheat flour at seven dollars a barrel, the twenty-five cents would pay for six and a quarter pounds of nutrients containing eight-tenths of a pound of protein and 11,755 calories of energy. Since it was accepted that the quality of each of the nutrients from each of

these foods common in the American diet was essentially the same, the logical course seemed to be to buy the cheaper articles when economy was essential. There was no fear that any diet would prove unsatisfactory provided it contained sufficient protein and sufficient available energy.

It was pointed out by Atwater that the price of food was not regulated solely by its nutritive value, but that agreeableness to the palate or to the fancy was a factor of great significance in determining the market price. There was no appreciation of the peculiar worth of certain foods as supplements to others until after the year 1900. Nor was there any appreciation of the difference in quality of foods such as we now fully recognize. The astonishing fact that certain lists of common foods do not maintain satisfactory nutrition, even though they have collectively a chemical composition exactly similar to those of another list which is satisfactory for the promotion of nutritive well-being, did not become appreciated until about 1915.

Physiological Economy in Nutrition.—It is the great merit of Professor Chittenden of Yale University to have introduced the idea of physiological economy in nutrition (1904-07). Numerous dietary studies in Europe and America had shown fairly accurately what the people in several countries were eating. The amounts and the chemical composition of the foods had been determined. The most notable students of this subject were Voit in Munich and Atwater in America. Voit came to the conclusion that an adult man weighing 70-75 kilos and doing moderate work requires a diet containing 3,000 calories of energy. He believed that it should furnish 118 gm. of protein daily, 56 gm. of fat, and 500 gm. of carbohydrate. Atwater's standard called for 120 gm. of protein. These standards were based upon statistical studies of large numbers of people. It was assumed that appetite and instinct were safe guides to physiological needs.

These standards were derived through studies of the actual consumption of food by families in various walks of life. Those who accepted them as the amounts of the various food components which the body requires under specific conditions, accepted the view that man, when in a position to select his food from a suitable variety such as exists in times of relative plenty and moderate values, would take under the guidance of the appetite the amounts of food which are best suited to his bodily needs.

Chittenden was the first to seriously question this doctrine of

instinctive selection. He began to experiment cautiously on himself to discover how low he could reduce the protein in his diet without interfering with his well-being. To his surprise he remained in nitrogen equilibrium and was improved in physical condition on a diet which contained only sufficient nitrogen of a digestible and absorbable nature to make the nitrogen content of the urine average 5.699 grams daily over a period of nearly nine months. This corresponds to about 36.72 grams of digestible protein. Chittenden felt convinced that this reduction of the intake of protein to approximately one-third of the accepted standard requirement for the human body resulted in marked improvement in physiological well-being. He determined to test this question on a scale which would afford convincing evidence, in order that mankind might profit by what was believed to be a great discovery, viz., that physiological economy in nutrition is a matter of great importance for the maintenance of strength and vigor.

The reasoning by means of which the good results of this abstemious diet, and especially of the low protein intake, were accounted for, is interesting. A diminished intake of protein leads to a decreased formation of crystalline waste products, such as acid potassium phosphate, uric acid and the purin bases and other end-products of nitrogen metabolism. These are in part responsible for the sensation of fatigue when they accumulate in the blood. The energy for work comes not from the degradation of muscle substance; it comes from the combustion of non-nitrogenous food-stuffs such as sugar and fat, and therefore it is unnecessary to take large amounts of protein food for the purpose of replacing the wear and tear quota, for the latter is but of small magnitude even in severe work. Protein stimulates metabolism in general, and hence will lead, when taken in excessive amounts, to a rate of metabolism far in excess of the normal amount which results from a diet lower in this factor. This augmentation in the metabolism of the tissues leads to a waste of energy-yielding substances which might otherwise be conserved.

It was noted that while on the low protein diet there was not a single day during the nine months when the urine was turbid, whereas on a rich protein diet it was necessary to take a large amount of water in order to prevent the urine from becoming concentrated. It was reasoned that this low protein regimen should relieve the kidneys of a burden and would

tend to aid them in maintaining their functional capacity unimpaired.

In order to demonstrate in a large way the importance of the new views, Chittenden secured the coöperation of a group of his colleagues in the faculty and a group of volunteer students. These men were allowed a free choice of food, but they adhered to the regulation imposed of selecting foods poor in protein. A group of soldiers of the United States Army were detailed under a sergeant to participate in the experiment. The diets provided these men were similar to those taken by the volunteers. The faculty and student groups ate little or no meat, which tended to lower the protein intake. In all cases there was free choice of food in the two groups.

There was no indication that the men were not in excellent physical condition at the end of the nine months on a diet abstemious both in energy and protein. The conclusion was drawn that the general standards of food consumption of people were faulty. The faults in the ordinary diet lay in the excessive consumption of food and especially of protein. The view seemed sustained that the body is better nourished when the amount of food metabolized closely corresponds with the minimum which is necessary to cover its requirements. Better nourishment was held to be due to relief of the digestive organs and the organs concerned with the transformation of protein. The liver, as well as the organs of excretion, the kidneys in particular, were relieved from an excessive burden to which the ordinary habits of man subject them.

There can be no doubt that the diets of these men did not lead to any observable deterioration in their well-being. Practically all maintained their body weights, or at least lost only amounts which would be anticipated as the result of the physical exercises which some of them took during the test. Their strength increased during the experiment where records were taken and all were said by their observers to be in a normal and healthy condition at the end of the experiment.

Parsimony in Nutrition.— Chittenden's conclusions were not accepted by many. A critical discussion of his conclusions was presented by Benedict (1906). He interpreted the digestion data obtained with the soldiers to indicate that the low protein intake had influenced adversely the absorption of protein. He further called attention to the fact that animals fed low protein diets do not thrive so well as on more liberal quantities. Benedict,

and also Crichton-Browne (1909), pointed out that dietary studies all over the world had shown that in civilized communities where productive power, enterprise and civilization are at their highest, man has instinctively and independently selected liberal rather than small quantities of protein.

Studies in Under-Nutrition.—In 1917, when there was a food shortage brought about by the World War, Benedict conducted an experiment on twelve students of a Y. M. C. A. College, to see what the effects would be of rapidly reducing the weight by about 10 per cent. by a sharp reduction in the amount of food ingested. This was accomplished in 4 to 6 weeks. Thereafter the food consumption was so regulated that the weight was maintained at the new level. Each man lost approximately 150 gm. of nitrogen, equivalent to about a kilo of protein. Their metabolism was reduced, therefore, to a distinctly lower level than normal during the greater part of a school year. From the results of his observations he was led to modify his former view and became a convert to the idea that the abstemious diet best promotes physical well-being (1918).

Experiments Not Planned to Give Conclusive Results.—The question arises: How far are we justified in drawing conclusions from these experiments as to whether this is a satisfactory regimen to which man may safely adhere throughout adult life? In the light of the new viewpoints gained by animal experimentation, it must be admitted that these results on men do not seem sufficiently convincing to warrant conclusions relative to the most important question which arises in the life of the individual, viz., that of maintaining to a late period in life the full vigor of youth.

These experiments on men covered approximately 1 per cent. of the normal span of life for men of their ages. It has been abundantly demonstrated on animals that diets which fall below the standard of quality necessary to maintain life and vigor over a very great fraction of the span which the species is capable of living, may cause no noticeable deterioration in physical condition, during even 5 per cent. of the normal expectation of life. In order to produce noticeable effects by faulty diet during 4 or 5 per cent. of the average life, it is necessary that the faults be relatively severe. However, diets which are just good enough to permit a young animal to develop in what appears to be an approximately normal manner, may cause early aging, low fertility, or instability of the nervous system. While growth is still

in progress, or after it has ceased, the adherence for even periods of a few days or weeks to a diet which is unsatisfactory in some degree, leads to a deviation from the normal histological structure of the osseous, the nervous or the circulatory tissues, depending upon the nature and extent of the faults in the food. Such abnormal modification of tissue structure would ordinarily escape observation even to-day, and certainly a few years ago.

Effects of Faulty Diets on Life Histories of Animals.—We may illustrate this principle by the results of animal experimentation. In the authors' laboratory a large group of rats were restricted to diets which were faulty in different degrees with respect to the quality of their protein. The experiments included the period of growth, and of adult life at least to the point of development of senile characters. Fertility and success in the nursing of young, the stability or instability of the nervous system as shown by indifference to being handled, which is characteristic of well-nourished rats, or the apprehensiveness and timidity of many malnourished ones, were carefully observed. Successive generations of the families, where young were reared, were maintained on the family diet. These experiments will be discussed more in detail later. The deviation of the quality of the protein moiety of the diet even in slight degrees from the optimum, exerted a profound influence on the *life history* of the animals and on the *functioning of the nervous system.*

It may be added that the faults of a diet need be no more pronounced than are encountered in a mixture consisting of cereal grains, legume seeds, tubers, fleshy roots and muscle meats, to produce profound physical deterioration when such a regimen is adhered to over any considerable period.

Experiments on Man Less Than Five Per Cent. of Span of Life.—The experiments of Chittenden and of Benedict covered but approximately 1 per cent. of the expectation of life of the young men who served as subjects. They can scarcely be used as a basis for deductions of a far-reaching nature concerning the dietary practices which man may safely adhere to throughout long periods. Our conclusions as to what is safe or unsafe must be derived from tests of special types of diets on animals. The life history must be carefully observed, and the vitality of the offspring compared with a satisfactory standard of excellence. The correlation of these results may be made with the experience of man in various parts of the world, where groups are found

whose dietary habits are of peculiar and restricted types. Such data will be set forth in later chapters.

Optimum a Better Goal Than "Normal" in Nutrition.—The studies of McCollum and Simmonds introduced the idea that the word "normal" is too indefinite a term. It is less satisfactory than "optimum" to designate the standard of excellence toward which we should strive. They emphasized that our standards of what constitutes normality are based upon common observation rather than the best possible achievement as illustrated by the best specimens in the population. They also pointed out that it is unsafe to judge by the appearance or feelings of a man or animal under special conditions during a relatively small fraction of life, especially that part in which recuperative power and endurance are greatest, that a dietary regimen which has proven unsafe for the promotion of vigor and health in animals is safe for man. Physical deterioration may not be recognized until it is pronounced. It is important to appreciate that in the aggregate it is borderline states of malnutrition, characterized by a condition of nutritional instability, that is the greatest menace to the individual when his health and efficiency over the life period are considered. A similar view has been recently expressed by Rose (1928).

With this attitude toward the subject, we may turn to some of the more technical phases of nutritional investigation. The results of these researches have served as a basis for practical conclusions of great value for the health of man.

CHAPTER III

THE BIOLOGICAL METHOD FOR THE ANALYSIS OF A FOOD-STUFF

Osborne and Mendel's Studies on the Purified Food Mixture.—It was pointed out in Chapter I that failure characterized experimental studies in which animals were confined to diets containing onl the well-known food principles. These failures had practically established the belief that this line of study was unprofitable, because all animals, for some unknown reason, died when confined to such a regimen. The moderately successful results of McCollum with such mixtures aroused hope that new data could be secured which would reveal the sources of valuable and less valuable proteins among our natural foods.

In 1909 Osborne and Mendel took up this line of investigation. They fed individual proteins with a diet which was otherwise composed of starch, lard and mineral salts. They planned to interpret on the basis of the capacity of young rats to grow on a constant diet, varied in successive experiments only with respect to its protein, the relative merits of such proteins as tissue builders. They did not, however, actually duplicate the experimental conditions of McCollum, but adopted a diet which they regarded to have essentially the same properties. This consisted of purified protein, starch, lard and a salt mixture. They met with complete failure in these attempts to nourish their animals. They (1911) tried a salt mixture recommended by Röhmann (1908) with which he had had success with a diet consisting in *great measure* of purified food substances; they also used the salt mixture described by McCollum. In all cases, however, their animals declined steadily from the time they were confined to such diets. They proceeded a step further, and established the fact that the failure was not due to lack of appetite, since the food intake was sufficient to promote growth, provided the composition of the food had been satisfactory. At that time no one was able to see any important difference between the quality of a purified protein, three carbohydrates, *one*

being milk sugar, two kinds of fats, *one being butter fat*, and a salt mixture, as compared with a simple mixture of protein, starch, lard and salts (McCollum, 1909).

"Protein-free Milk."—Since Osborne and Mendel failed to nourish rats on a diet of isolated and purified nutrients, they adopted what they apparently regarded as essentially the equivalent of a mixture of purified food-stuffs. The basis of their new diet was 28 per cent. of "protein-free milk." This product was made by removing as completely as possible the fat, casein and lactalbumin from milk, and evaporating the resulting whey to dryness. The residue was a yellow solid, easily powdered. It consisted for the most part of milk sugar and the mineral salts present in the milk. In fact, no other components of the product could be named. "Protein-free milk" was found to contain about 0.7 per cent. of nitrogen, but the form in which it was present could not be ascertained.

They fed diets consisting of 28 per cent. of this product, the other components being starch, lard, agar-agar, and the protein they desired to study. This combination had certain properties which could not be accurately defined, but which made it far superior to any of the simple mixtures used by others. With these diets they were able *with certain proteins* to secure in rats normal growth to the full adult size. The results were regarded by many as the successful accomplishment of the quantitative comparison of the nutritive values of purified proteins. A more detailed account of their protein studies is given in the next chapter.

"Artificial Protein-free Milk."—Since Osborne and Mendel found their diet containing "protein-free milk" suitable for the purpose which they had in view, viz., the comparison of the relative values of various isolated proteins, they apparently abandoned further efforts to determine what was lacking in their former diets of purified food-stuffs. They did prepare an "artificial protein-free milk" from what they regarded as pure milk sugar and a mixture of mineral salts made up from reagents. An attempt was made to imitate exactly the composition of "natural protein-free milk." With this mixture in place of the natural product, they secured excellent growth records over periods covering about two months (1913). They made a special effort to secure lactose and salts of a high degree of purity for certain of their experiments in which "artificial protein-free milk" was used. This preparation gave negative results in most

cases, and in all the experiments growth was decidedly less than when materials of ordinary purity were used. They carried their investigations in this direction no further than to make additions of traces of iodine, manganese, fluorine and aluminum. These impurities, suspected in their reagents of poorer quality, improved the diet, making it capable of inducing better growth, but the rats remained under-sized. They were inclined to accept the view that the peculiar virtue of their natural "protein-free milk" lay in the nice adjustment of the inorganic elements it contained.

Studies of Purified Food Mixtures Confusing.—There was no apparent explanation for their failure. The diet on which McCollum had reported growth consisted of two pure proteins, several carbohydrates, including milk sugar, two fats, including butter fat, and a salt mixture (p. 33). McCollum had erroneously supposed that the success observed with this mixture was due to palatability and consequent adequate food consumption. An effort was at once begun by him to discover wherein lay the lack of harmony in these observations. It was imperative that he should satisfactorily explain why others had failed to confirm his results. On returning to this field of study he found his results irregular and confusing. Many experimental diets were fed before a clue was uncovered which finally led to the discovery of the true explanation of his results.

Osborne and Mendel's Fat-free Diet.—Osborne and Mendel (1912) published a paper describing successful nutrition for about 120-160 days with diets which contained no fats and only traces of substances soluble in fat solvents such as ether. The diet consisted of protein, cane sugar, starch and "protein-free milk." In some experiments with similar diets "artificial protein-free milk" was used. The artificial product was composed of milk sugar and a salt mixture from reagents. It was made in close imitation of the mineral content which analysis showed to be present in the natural product.

In commenting on these results, Osborne and Mendel stated: "Employing the methods which were adopted in our earlier feeding experiments with isolated food substances, we have succeeded in inducing a normal rate of growth in white rats with dietaries devoid of fat throughout almost the entire period during which growth ordinarily continues." They stated that "McCollum has demonstrated that the phosphorus needed by an animal for phosphatid formation can be drawn from inorganic

phosphates, and that phosphatids can be synthesized anew in the animal body. Röhmann asserts the possibility of lecithin synthesis in mice which were maintained into the second generation on lecithin-free food. Our own experiments point in the same direction with regard to the lipoids in general, and they give positive evidence of the dispensableness of true fats for growth."

It appears that they did not regard their failure with purified diets as due to the absence of accessory factors. This may be assumed from their statement that they had achieved a considerable degree of success in the absence of the hypothetical vitamins. Hopkins and Neville (1913) challenged their statement and attempted to repeat their experiments. Using carefully purified ingredients they were unable to obtain any appreciable growth on such diets. The animals, in spite of satisfactory consumption of food, were all dead before the expiration of 40 days. In confirmation of the earlier experiments of Hopkins it was found that when 2 c.c. of milk, per day, was given, in addition to the basal diet, this failure was avoided.

Unique Dietary Property of Butter Fat. Discovery of Vitamin A.—When Osborne and Mendel's paper appeared, McCollum and Davis had already accumulated experimental data which led them to believe that there were great differences in the nutritional value of fats from several sources. With diets composed of protein, starch, milk sugar, salts and fats, it had been found that prolonged growth was secured when butter fat was added. The butter fat had been dissolved in ether and passed through filter paper in order to make certain that no traces of any ingredients of the milk except fats and substances having the same solubilities were present. Egg yolk fats behaved like the purified butter fat, whereas little growth and early failure resulted when olive oil or lard were the only fats in the diet. Due to an epidemic which destroyed the rat colony, a year elapsed before a demonstration of this fact could be secured. McCollum and Davis (1913) recorded their findings that purified butter fat, and likewise egg yolk fats, contain something which greatly promote growth, and that lard and olive oil did not possess this property. This "something" is now known as Vitamin A.

Synthesis of Complex Lipins by Animal Body.—McCollum and Davis appreciated the fact that the animal body is capable of producing synthetically the complex fat-like substances such as lecithin and kephalin, which in addition to glycerol and fatty

acids contains phosphoric acid and the nitrogenous base choline. McCollum, Halpin and Drescher (1912) had shown that young hens could increase their weight 35 per cent. on a diet free from this class of substances. While confined to it hens produced many eggs in which were contained much more of the complex lipins than the bodies of the hens could furnish. The birds were in good health at the end of the experiment. Since butter fat is free from lecithin and related substances, McCollum and Davis interpreted their data to mean that the stimulating effect of butter fat on growth could not be attributed to any known lipin.

Osborne and Mendel's Observation on Butter.—Osborne and Mendel had discovered by this time the error of their conclusion that growth and well-being could be secured in animals restricted to a diet free from fats and containing "but an insignificant trace of ether extract." The month following the appearance of the paper by McCollum and Davis (1913) demonstrating the peculiar value of butter fat and egg yolk fats as contrasted with lard and olive oil, Osborne and Mendel (1913) described the preliminary period of growth they secured with a diet of purified protein, starch, sugar and "protein-free milk"; this short period of growth, which extended between 60 and 120 days, was followed by decline. The decline could be checked by the addition of 16.4 per cent. of *butter*. Animals which received the butter from the beginning of the experiment grew uninterruptedly to maturity. In their paper on nutrition with fat-free food-stuffs, they had interrupted their experiments at the end of about 140 days from the time when the animals were restricted to the "protein-free milk" and "artificial protein-free milk" mixtures.

It was not possible to draw any conclusions from the data presented in their paper now under discussion as to whether the unknown food complex present in butter was in the fat or the non-fat fraction of the latter. Butter contains about 15 per cent. of milk substance other than fat. This portion consists, among other things, of water, protein, cells from the mammary gland, leucocytes, and bacteria. It was impossible to decide without further evidence whether it was in some of these or in the fat itself that the substance occurred which exerted such remarkable effects on the growth of animals. Hopkins (1912) had reported that rats restricted to diets of purified food-stuffs steadily declined, whereas similar ones which were confined to the same diets, but in addition received 1 to 3 c.c. per day of fresh milk,

CHART I

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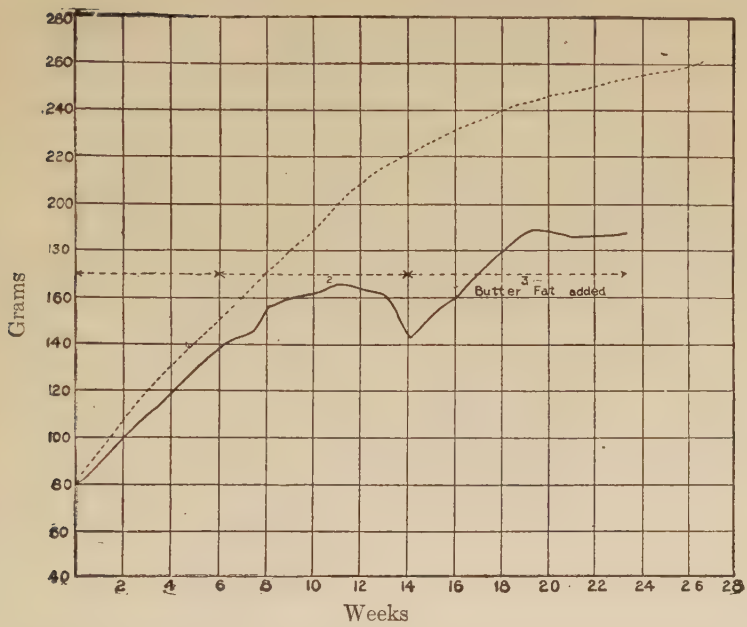


CHART I.—(*Rat* 141, *male*) shows the record of a rat which grew continuously, although slightly under normal rate, during 80 days on a ration of relatively pure food substances. There was at this time a rapid decline in body weight. The addition of 10 per cent of ether-soluble butter fat to the diet led to a prompt resumption of growth during the following 35 days, when the rat gained 50 gm. The rations employed were as follows:

PERIOD I	*per cent*	PERIOD II	*per cent*	PERIOD III
Salt mixture	6	Salt mixture	5	Same as II with butter fat replacing part of dextrin.
Casein	12	Casein	12	
Lard	20	Lactose	20	
Lactose	15	Dextrin	61	
Starch	42	Agar-agar	2	
Agar-agar	5			

The salt mixture employed consisted of:

	grams		*grams*
Sodium chlorid	0.61	Calcium lactate	11.38
Dipotassium phosphate	17.00	Magnesium citrate (10.2% Mg.)	23.42
Monocalcium phosphate	1.63	Ferric citrate	1.00

The lactose in the diet was not entirely pure; it contained enough of vitamin B to support growth.

The curve in Chart I represents the original growth record published by McCollum and Davis, which led them to the discovery of the special growth-promoting properties of butter fat as contrasted with vegetable fats and certain animal fats.

were able to grow. Even after decline had set in on the basal diet it could be checked by this small quantity of milk, which in no instance exceeded 4 per cent. of the dry weight of the diet.

Osborne and Mendel's Studies on Butter Fat and Cod Liver Oil.—Five months later Osborne and Mendel (1913) described experiments analogous to those of McCollum and Davis. They fed their animals protein, starch, "protein-free milk," lard, and *butter fat.*

It is now clear that the reason for the better growth of Osborne and Mendel's animals when butter fat was added to the diets, was the fact that their "protein-free milk" contained an abundance of a second dietary factor, the antineuritic substance which prevents beri-beri, whereas the diets of McCollum and Davis contained decidedly less of this substance which was present as an impurity in the supposedly pure milk sugar which was included in their diets.

Osborne and Mendel (1914) confirmed the observation of McCollum and Davis that egg yolk fats had the same effect on growth that butter fat had, and that lard did not stimulate growth as did egg and milk fats. They investigated almond oil and found it to resemble olive oil in that it did not promote growth. They further demonstrated that cod liver oil contained the dietary principle found in butter fat and egg yolk fats.

Indispensability of Certain Lipins.—The results of these investigations recalled that in 1909 Stepp had described experiments in which he had found that bread prepared with milk was capable of maintaining adult white mice without loss of weight for an indefinite period. When the same bread was extracted with alcohol-ether, the animals restricted to it rapidly declined. Replacement of the extracted material failed to restore the properties of the original bread in those cases where the extracts had been heated for several days. When care was exercised to avoid high temperature or prolonged heating, the addition of the extracts made the extracted bread a complete food. Stepp later showed that the addition of the salts which were extracted with the lipoids did not prevent decline of the animals. *Butter additions did not prevent decline,* but the addition to the extracted bread of lipin extracts of egg yolk, bran, dry milk or wheat, restored the extracted bread to dietary completeness. This result led Stepp to suggest the existence of an unidentified indispensable dietary unit, which he appears to have regarded as a

member of the lipoid class. He was unable to identify this with cholesterol, lecithin, kephalin, and cerebron. All members of that class of substances failed to restore the nutritive value of the extracted food-stuffs. He states, "It is not impossible that unknown substances indispensable for life go into solution with the lipoids, and that the latter thereby become what may be termed carriers for these substances."

Confusion Concerning the Essential Nutritive Factors Previous to 1915.—It has been pointed out that students of normal nutrition failed to make use of the observations of the pathologists during the decade 1905 to 1915. In discussing the possible explanation of the early failure of their animals fed isolated food-substances, together with "artificial protein-free milk," Osborne and Mendel (1913) emphasized the improvement of the latter by the addition of traces of manganese, iodin, fluorine and aluminum, and dwelt upon the possible importance of minor variations in the inorganic moiety of the diet. Even at the end of 1913, in commenting upon McCollum's studies of butter fat, they stated: "The added butter fat may have simply supplied something analogous to the so-called vitamines, which Funk considers to be essential for life, and thereby enabled the animals to resume growth on a food thus made adequate for maintenance." Further, "It is still rather early to generalize on the rôle of accessory 'vitamines' when the ideal conditions in respect to the familiar fundamental nutrients and inorganic salts adequate for prolonged maintenance are not completely solved." These quotations will serve to show the state of confusion as to the interpretation of the data in the literature of pathology which described the experimental production of beriberi and of scurvy.

Biological Method for Analysis of a Food-stuff.—It is easy now to see in the light of later experience why such skepticism existed regarding the necessity of hitherto unappreciated factors in the diet. Osborne and Mendel, and McCollum, had seen young rats grow for a considerable time when restricted to diets of supposedly pure food substances. These diets, however, always contained a considerable amount of milk sugar, and it was not appreciated at the time that impurities of a most important nature might still adhere to lactose of a relatively high degree of purity.

When it was found that the addition of butter fat promoted growth on a diet which consisted, aside from this fat, only of

substances of accepted purity, every component of the diet being regarded as known chemically, it seemed very plausible that the only unidentified dietary essential for the rat was associated with the fat fraction. This view was at one time held by McCollum. In all such studies the question as to the degree of purity of the ingredients of the diet necessary to warrant the conclusion that no impurities of a significant nature were present could be decided only through critical and comparative experiments.

The first advance in solving the problem concerning the number of unidentified nutrient principles essential in the diet of the rat is of historical interest. During the years 1913 to 1918 Osborne and Mendel, and McCollum and his coworkers confined their researches to different fields. The former, using a diet containing a protein and other isolated food-stuffs, supplemented with 28 per cent. of "protein-free milk," extended their investigations in the direction of determining the biological value of each of the more important proteins isolated from natural foods. McCollum and his coworkers turned their attention to the study of the deficiencies of our natural foods from the dietary standpoint. It was through the latter studies that the next advance was made in our knowledge of what constitutes a satisfactory diet.

While the studies with cattle fed rations restricted to a single plant source were in progress at the Wisconsin Experiment Station, McCollum, beginning in the year 1907, restricted the diet of rats to each of the more important single grains as the sole source of nutriment. Whole wheat, rolled oats, maize, or any other seed fed singly, failed to induce any growth in young animals, or to maintain life for a long period. It seemed in 1913 after the completion of the experimental studies described that the necessary knowledge was available to enable one to determine the nature of the faults responsible for these failures.

Chemical Analysis versus Biological Analysis of Food-stuffs. —Chemical analysis showed the cereal grains to contain all the essential food substances for which we know how to analyze. *It was assumed as a working hypothesis that the only unknown factor contained in the diet was that associated with certain fats.* The first study was made on the wheat kernel. It was reasoned that since all the types of nutrients are represented in a seed such as wheat, except possibly the unknown one which had been demonstrated in certain fats, the fault or faults of

wheat from the dietary standpoint must lie in the *quality* of one or more of these food factors. It was believed that valuable data might be secured by enhancing the protein, the inorganic moiety and the fat factors separately in feeding experiments; and that by a systematic series of feeding experiments one would discover which was the limiting factor for growth in this seed. This possibility was investigated by enhancing the quality of one nutrient principle at a time. This procedure has since been known as the biological method for the analysis of foodstuffs. Accordingly, McCollum and Davis in 1914 fed the wheat kernel in the following combinations, with the results noted:

1. Wheat alone: No growth, short life.
2. Wheat plus purified protein: No growth, short life.
3. Wheat plus a salt mixture which gave it a mineral content similar to that of milk: Very little growth.
4. Wheat plus a growth-promoting fat (butter fat): No growth.

From these results it seemed apparent that either the working hypothesis concerning the factors which are necessary in the diet must be wrong, or there must be more than a single dietary factor deficient in wheat, and jointly responsible for the poor nutrition of the animals. Another series of experiments was carried out, in which wheat was supplemented with two purified food additions:

5. Wheat plus protein, plus the salt mixture: Good growth for a time. Few or no young. Short life.
6. Wheat plus protein, plus a growth-promoting fat (butter fat): No growth. Short life.
7. Wheat plus the salt mixture, plus a growth-promoting fat (butter fat): Fair growth for a time. Few young. Short life.

The behavior of rats fed wheat with two purified food additions suggested that there were three dietary factors of poor quality in the wheat kernel. This was demonstrated to be true by a feeding trial in which wheat was fed, supplemented with three purified food additions:

8. Wheat plus protein, plus the salt mixture, plus a growth-promoting fat (butter fat): Good growth, normal number of young, good success in rearing young. Life approximately the normal span. (See Chart II.)

These results seemed convincing to McCollum and Davis that the only element of mystery in the normal diet was the unidentified substance in butter fat. With the improvement of three

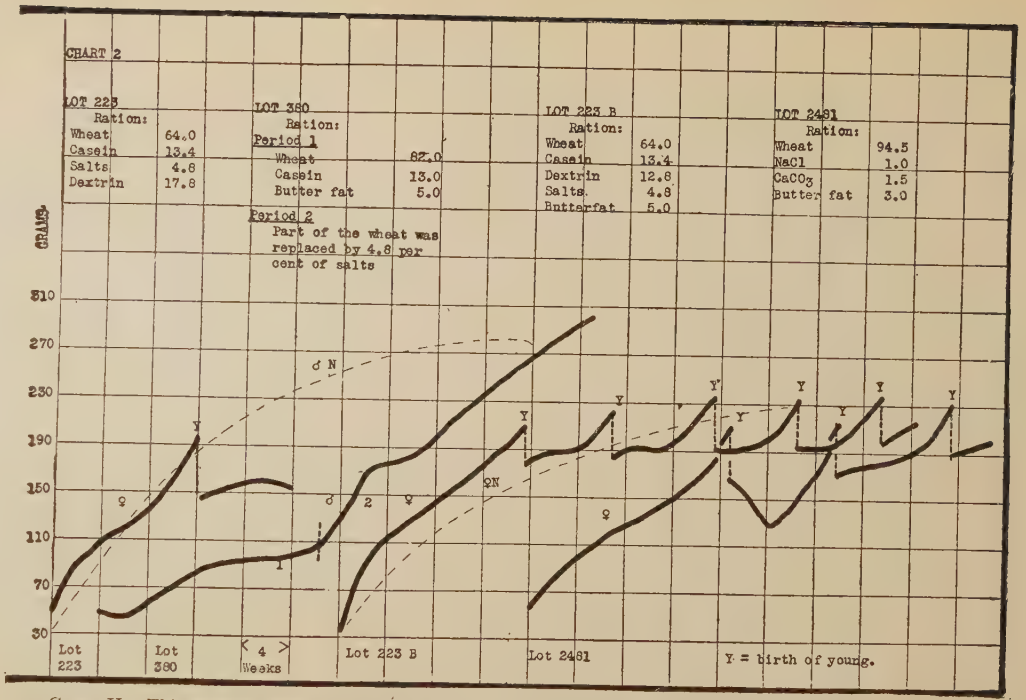


CHART II.—This series of curves illustrate the nature of the deficiencies of the wheat kernel from the dietary standpoint. Lot 223 lacked sufficient vitamin A but was otherwise complete. Lot 380 contained everything which was essential except certain mineral salts (Period 1). In Period 2 the animals became able to grow because of the inclusion of the missing calcium, sodium and chlorin. Lot 223 B grew well and were very fertile and successful in rearing young on wheat supplemented with respect to protein, mineral salts and vitamin A (in butter fat). The record of the animal on ration 2481 shows clearly that the mineral deficiencies of wheat are most serious, for without enhancing the proteins of this cereal, good growth and moderate fertility were secured. This curve further shows that only a calcium salt and common salt or sodium chlorid are necessary to complete the inorganic moiety so as to make possible fairly satisfactory nutrition.

dietary factors, wheat became a satisfactory food for nutrition during growth, and for support of the functions of reproduction and rearing young.

These experiments brought to light two new viewpoints in nutrition. First: **Although the inorganic content of the wheat kernel furnishes all the necessary elements, it does not contain enough of certain of these to meet the requirements of a young animal during growth.** Henry (1889) had called attention to the deficiency of the corn kernel in ash content and had added wood ashes in some experiments. He noted improvement in the well-being of the animals. The fact that a seed, such as wheat, failed to supply sufficient inorganic elements was not generally appreciated. Later work by McCollum and Simmonds (1917-1921) demonstrated that the deficiency in mineral elements in wheat and other seeds is limited to the four elements, viz., calcium, phosphorus, sodium, and chlorine. Second: **The wheat kernel is too poor in its content of the unidentified substance which butter fat contains to nourish an animal satisfactorily.**

Deficiencies of Polished Rice.—The above investigations were carried out in 1913, soon after the publication by Funk of his work on polyneuritis. In 1912 Hopkins had called attention to the remarkable effects of adding small amounts of milk to diets of purified food-stuffs. The vitamin hypothesis had just been formulated by Funk (1913). McCollum and Davis were aware, therefore, of the relation of a diet of polished rice to experimental beri-beri. They believed, in the light of their experience with the diet of purified protein, milk sugar, fats and inorganic salts, that such a mixture was capable of inducing growth when certain fats were supplied, but not with others. The further fact that wheat could be supplemented by purified protein, a growth-promoting fat, and a suitable salt mixture, seemed to indicate that there was but a single unidentified substance necessary in the diet. They applied to polished rice the same procedure which had shown so clearly the nature of the dietary deficiencies of wheat. They reasoned that rice could be nothing less than a mixture of proteins, starch, fat and inorganic salts, similar to that contained in wheat, but in different proportions, and that polished rice could be supplemented with a suitable salt mixture, a purified protein and a growth-promoting fat, and thereby induce growth and maintain animals in health. This seemed to be a necessary corollary, since they had secured growth and

well-being in animals fed solely upon a mixture of purified protein (casein), starch, milk sugar, butter fat, and a mixture of inorganic salts of suitable composition (1913).

It was a great surprise to McCollum and Davis to find that polished rice supplemented with the protein, casein, butter fat, and a properly constituted salt mixture, failed to induce growth (1915). Not only did rats fail to grow, but also in a few weeks in some cases they developed a state of paralysis suggestive of polyneuritis. Here was an apparent contradiction. The polished rice was nothing less than a mixture of protein, carbohydrate, fat, and salts. The only difference between this mixture and the one of supposedly purified food-stuffs with which they had observed growth was in the 20 per cent. of milk sugar which the synthetic diet contained.

Demonstration of the Existence of Vitamin B.—They therefore repeated the experiments with the latter mixture, with the milk sugar replaced by starch. It was found that this change in the food mixture made the difference between success and failure. No growth could be secured when the milk sugar was omitted. Later experiments showed that if milk sugar sufficiently purified by repeated crystallization was added to the purified food, it was no longer effective in inducing growth, whereas the water from which the sugar had been crystallized would, when evaporated upon the food mixture, render it capable of inducing growth. This made it evident that there is a second dietary essential, of which an animal needs but a very small amount, but which is necessary for growth in the young and for maintenance of health in the adult. This second dietary essential is now known as vitamin B.

Further experiments were then conducted to find whether this unidentified substance which was being added accidentally as an impurity in the milk sugar was the same as the one which Funk had discovered in his studies of beri-beri. It was found that pigeons which had developed beri-beri as the result of being fed exclusively upon polished rice could be "cured" with any preparation which would cause animals to grow when it was added to a diet of purified food-stuffs containing a growth-promoting fat. See Chart III.

McCollum and Davis in 1914 followed the method of Funk and Suzuki of employing alcoholic extracts of various natural foods. They added the alcohol-soluble matter to the standard mixture of protein (casein), starch (partly dextrinized), salts

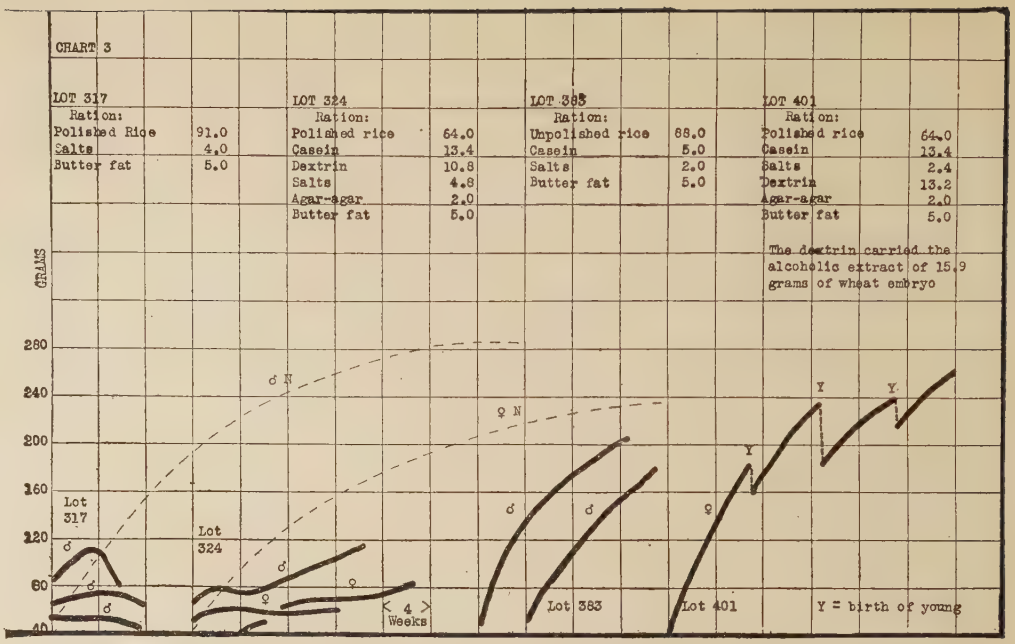


CHART III. The four groups of curves illustrate the nature of the deficiencies of the polished rice kernel as a food for the rat. Lot 317 could not grow on rice, a complete salt mixture and butter fat because the anti-neuritic substance vitamin B was absent. Improvement in the protein by the addition of casein did not enable the animals to grow except to a very slight extent. Unpolished rice contains the germ which is rich in vitamin B, so the addition of the same supplements as in Lot 317 served to complete it and make a diet on which growth was rapid. Lot 401 differed essentially from 324 in that it contained the alcoholic extract of wheat embryo or germ. This supplied vitamin B, and therefore enabled the animals to grow, since all other deficiencies of polished rice were made good by the addition of a protein (casein), a complete mineral salt mixture and butter fat which contained vitamin A.

and butter fat, and became convinced that the substance which relieves polyneuritis in pigeons was always present in the preparations which rendered the diet of purified food-stuffs capable of promoting growth. An alcoholic extract of ether extracted wheat germ was finally adopted as a source of this dietary factor in later investigations. Funk and his coworkers had shown that the curative substance is present in many natural foods (1915). Repeated experiments by McCollum and Davis showed that the inclusion of the alcoholic extract of wheat germ, or of other food was insufficient to induce growth unless butter fat or other similar fat was likewise added to the purified food mixture. Both the growth-promoting fat and the trace of unidentified substance in the alcoholic extract of wheat germ are necessary for the promotion of growth or the preservation of health (1915).

Funk and Macallum found butter fat to be ineffective for the cure of polyneuritis in pigeons, and accordingly considered it free from "vitamine." They fed young rats a diet of purified food-stuffs supplemented with a liberal amount of butter fat, and observed that the animals did not grow. Although this result was due to the lack of an antineuritic substance, no one at that time was clear as to the several factors which are necessary in the diet; these workers interpreted the data as evidence that butter fat possessed no growth-promoting properties (1915).

Observations on Lipin Extracts.—Stepp was unable to detect any special dietary property in butter fat. He showed that alcoholic extracts of certain natural foods, e.g., egg yolk, bran, and dry milk, possessed remarkable properties in that they completed from a nutritional standpoint his lipoid-free bread and milk diet. This is now readily understood. His alcoholic extracts contained some of both the uncharacterized substances discussed above, whereas butter fat contains but one. The latter, without a supplementary source of a second dietary factor, would permit of failure of the animals.

Vitamin Hypothesis "In the Air" in 1914.—From what has been said of the experimental studies which ultimately led to the conception that there were necessary two uncharacterized dietary essentials, in addition to the long recognized food principles in the diet of the rat, it will be appreciated that about 1914-15 there were suggestions of various kinds "in the air," and that the time had arrived when some of the several investigators working in the field would soon formulate, on the basis of definite

experimental evidence, a working hypothesis concerning the essentials of an adequate diet. Thus Mendel, in his Harvey lecture, November, 1914, said: "It is not unlikely—to speak conservatively—that there are at least two 'determinants' in the nutrition of growth. . . . Without this 'determinant' . . . the special components of butter fat or cod liver oil or egg fat induce only limited gains at best. Another 'determinant' is furnished by these natural fats. Either of the 'determinants' may become 'curative,' both are essential for growth when the body's store of them (if such there be) becomes depleted. It is too early to attempt a tenable conclusion."

Essential Components of Adequate Diet.—As a result of their studies, McCollum and Davis formulated in 1915 their working hypothesis of what constitutes an adequate diet. In addition to the long recognized dietary factors, viz., (1) protein; (2) a source of energy in the form of proteins, carbohydrates or fats; (3) a suitable supply of certain inorganic salts, they stated that it must also contain two as yet unidentified substances or groups of substances. One of these unidentified substances is associated with certain fats, and is especially abundant in butter fat, egg yolk, cod liver oil, and the fats of the glandular organs such as the liver and kidney, but is absent, or present in but traces, in fats or oils of vegetable origin. The second substance is never associated with fats or oils of either animal or vegetable origin. It is widely distributed in natural foods. It can be obtained in a concentrated form but has not been prepared in a state of purity. It is soluble in water and dilute alcohol; these extracts always contain the substance which cures polyneuritis in birds. These unidentified substances are now called vitamins A and B.

This hypothesis contained all that could be said about the needs of the rat for dietary essentials hitherto unappreciated at that time (1915). Later it became evident through the studies of Holst, Chick, Mendel, and others, that man and the guinea pig require a third substance, now known as vitamin C, which is not necessary in the diet of the rat. Studies on bone growth as influenced by diet have demonstrated that another unidentified factor vitamin D is necessary for the normal development of bone (Chap. XVIII). Evidence has also been adduced which demonstrates that a substance called by Evans and Bishop "Vitamin X," now vitamin E, is necessary for the normal reproduction of the rat. Benedict and Sugiura (1919) have reported

observations which they interpreted to indicate that a diet can be adequate for the production of young and for growth after the eyes are opened, but inadequate for the proper production of milk; these results suggest the necessity of some specific principle for milk secretion.

Nomenclature of Vitamins.—The nomenclature of the newly discovered nutrient principles is more or less in a state of confusion. Stepp (1909), regarded them as lipins. Funk (1913) designated as "vitamines" a hypothetical group of protective substances of unknown nature. Hopkins (1912), after discovering the remarkable growth-promoting effects of small additions of milk to a diet of purified food-stuffs, termed them "accessory" food substances.

Bottomley (1914) designated as "auximones" a group of substances which he believed stimulated plant growth. Wildiers in 1901, long before the formation of a conception of the vitamin hypothesis, observed that when a synthetic medium containing only well-defined substances was inoculated with yeast, no growth occurred unless the inoculation was a large one. He explained the phenomenon by assuming that the yeast required for its development some unknown substance which was not present in a synthetic medium, but was found in beer wort and in all growing cultures of yeast. He gave the name "bios" to this substance, and investigated its properties. The oldest name "x-Saure" was proposed by Hulshoff-Pol to designate the antineuritic substance (1910). Suzuki and Shimamura called the substance curative for beri-beri "Aberisaure." Funk gave it the name "vitamine." Various commercial preparations have received such names as Oryzanin, Torulin, Orypan, Antiberiberin, Oridin, Sitacoid, Eutonin, Nutramin, and Vitavose.

When it was apparent that there were two unidentified dietary factors necessary in the nutrition of the rat, one soluble in fats, the other never associated with fats but easily dissolved out of natural foods by water or dilute alcohol, it became necessary to differentiate between them by names which would characterize them. None of the terms in use seemed desirable, so McCollum and Kennedy (1916) suggested the provisional use of algebraic terms, using a prefix designating characteristic solubility. They proposed the term fat-soluble A and water-soluble B respectively to designate them; these terms found widespread acceptance.

Upon the establishment of the existence of an antiscorbutic substance, Drummond (1919) designated it water-soluble C. He later (1920) suggested that the spelling of vitamine be changed to vitamin, and that the term be adopted to designate the entire group of chemically uncharacterized dietary essentials. This is in accord with the nomenclature of the hormones and the alkaloids, and indicates nothing of their chemical natures.

Gradually the terms vitamin A, B, C, D, E and G have come into general use and seem to be the most desirable from the standpoint of brevity. Recently, several investigators have shown that what we have been calling vitamin B consists of at least two and perhaps more indispensable principles, one related to polyneuritis, and another to pellagra. In addition, Evans and Burr (1928) have asserted their belief that their experimental work can be interpreted only by assuming that still another vitamin in some way associated with growth has hitherto been unwittingly added in the components of the ordinary "purified diet." Certain investigators have declined to follow the general usage of terms for vitamin nomenclature, and have devised their own nomenclature. The result is serious confusion. At the present time two committees representing English and American bio-chemists are attempting to agree upon a system which shall be adopted as official by their national organizations.

The committee on vitamin B nomenclature of the American Society of Biological Chemists have voted to make the following recommendations:

1. That the term "Bios" as suggested by the British workers, be retained to denote the factor or factors encouraging the rapid growth of yeast cells.

2. That the term "B" be restricted to denote the more heat-labile (antineuritic) factor.

3. That the term "G" be used to denote the more heat-stable, water soluble, dietary factor, called the P-P (pellagra-preventive) factor by Goldberger and associates, and which also has to do with maintenance and growth.

4. That the naming of newly discovered dietary factors, by other than descriptive terms, should be discouraged until their identity is established beyond question.

In Table I will be found nomenclature in use by different workers.

TABLE I

NAMES APPLIED TO VITAMIN B

Investigator	*Substance Curing Polyneuritis in Pigeons*	*Substance Promoting Growth in Rat*	*Thermostable Substance or Pellagra Preventive*	*Substance Accelerating Yeast Growth*
Schaumann	Activator			
Funk	Vitamine			
Suzuki et al	Oryzanin			
Tsuzuki	Antiberiberin			
Edie et al	Torulin			
Peters	Torulin*	Nutramine †		
Abderhalden and Schaumann	Eutonine			
Hofmeister	Antineuritin Oridin			
McCollum and Kennedy	Water-soluble B	Water-soluble B		
Drummond	Vitamin B	Vitamin B		
Funk; Jansen	Antiberiberi vitamine			
Funk				Vitamin D
Burrows and Jorstad				Archusia‡
Williams, R. J.	Beriberi preventing vitamine	Beriberi preventing vitamine		Beriberi preventing vitamin ⇔ Bios
von Euler		hDr		hDm
MacDonald-McCollum	Vitamin B	Vitamin B		Bios
Fulmer-Nelson				
Mitchell	Antineuritic vitamin	Growth promoting vitamin-water soluble A		
		Complex		
Goldberger et al	Vitamin B ⇄	Water-soluble B	⇆ Factor P-P	
Salmon	Vitamin B-P ⇄	Vitamin B	⇆ Factor P-P	
Chick and Roscoe	Antineuritic vitamin ⇄	Water-soluble vitamin	⇆ Vitamin B	
Sherman and Axtmayer	Vitamin F ⇄	Vitamin B complex	⇆ Vitamin G	
Accessory Factor Committee	Vitamin B_1 ⇄	Vitamin B	⇆ Vitamin B_2	Bios.
Funk	Vitamin B		Vitamin P (?)	Vitamin D
American biochemists' adoption	Vitamin B →	←	Vitamin G	Bios

* Peters uses the name torulin for substance curing head retraction in pigeons; and suggests that it may be different from Jansen's antiberiberi vitamin.

† Abderhalden and Schaumann refer to the unknown substances which make a diet biologically complete. The pigeon was their test animal.

‡ Burrows and Jorstad designated archusia as the non-specific substance necessary not only for growth but also for all activities of a cell. Tissue culture served as a test organism.

CHAPTER IV

THE PERIOD OF ORIENTATION IN NUTRITION STUDIES

Osborne and Mendel's Comparative Studies on Proteins.—Since 1909 numerous experiments have been conducted with a view to determining the relative nutritive values of a large number of individual proteins. It has been pointed out in Chapter III that Osborne and Mendel first sought to make comparative studies of isolated proteins by adding them to a mixture of purified carbohydrates, fats, mineral salts and water, and invariably met with failure. After their failure with the diet composed entirely of purified food substances, every component of which was known, they began a series of studies with what they believed to be essentially the equivalent of a purified food mixture. This involved the inclusion in the diet of about 28 per cent. of a product which they called "protein-free milk."

"Protein-free milk" was prepared by acidifying with acetic acid milk which had been freed from fat by centrifuging. The resulting whey, after the removal of the casein which flocks out on the addition of acid, was next heated to boiling to coagulate the lactalbumin. Casein and lactalbumin are the principal proteins of milk. Their removal extracts about 93 per cent. of the total nitrogen contained in the milk. The whey which was filtered from the lactalbumin precipitate was evaporated to dryness, leaving a yellowish residue consisting of milk sugar, most of the salts of the milk, and a certain amount of nitrogenous compounds for the separation of which no method is known. The final product which they included in their diets contained about 0.7 per cent. of nitrogen. This would be equivalent, if it were all in the form of protein, or of its digestion products, to 4.37 per cent. of the food mixture. It is well known that some urea, purines, creatine, and other simple nitrogenous substances which circulate in the blood are always found in milk. It is certain, however, that there is a considerable amount of nitrogen in "protein-free milk" in forms yielding amino-acids in the body.

Its property of enhancing certain incomplete proteins makes this view necessary.

Osborne and Mendel felt justified by the work of Munk (1893) in assuming that the 0.7 per cent. of nitrogen in "protein-free milk" was not a disturbing factor in their experimental studies. Twenty years previously Munk sought by coagulation and by precipitation with various reagents, to determine the amount of "non-protein" nitrogen in milk. He arrived at the conclusion that one-fifteenth of the total nitrogen in milk is in forms other than protein. Osborne and Mendel accepted this figure and reasoned as follows: A certain amount of milk contains a known amount of nitrogen, a part of which is in the form of proteins and can be removed by the methods employed. On evaporating the resulting whey to dryness the "protein-free milk" which remains contains 0.7 per cent. of nitrogen in forms not known. From the weight of the "protein-free milk" obtained, the total nitrogen it contained was calculated. From this amount they subtracted one-fifteenth of the total nitrogen contained in the milk with which they started. The remainder was assumed to represent "protein" or nitrogen having a nutritive value. There is no evidence in Munk's data, or in any furnished by later investigators, to show that the one-fifteenth of the total nitrogen of milk which remained after the removal of the casein and lactalbumin, and which was disposed of by mathematical treatment of their analytical data, was not a source of amino-acids.

The diet used in their studies, containing 18 per cent. of added protein in purified form, together with 28 per cent. of "protein-free milk" would derive 93 per cent. of its total nitrogen from the former and 7 per cent. from the latter source. The biological evidence appears convincing that this 7 per cent. of the nitrogen of their diets supplemented in a very effective manner certain proteins with which it was fed, and led in some instances to erroneous conclusions. Drummond (1916) commenting on these early studies said, "Osborne and Mendel have always been careful to utilize throughout their extensive researches proteins of a very high degree of purity, and yet they have gone to the other extreme in continuing to use in their diet 'protein-free milk.'"

Limiting Amino-acids in Proteins.—From what has been said in Chapter III it will be clear that a protein which is of low biological value for growth because of the fact that one or more of the digestion products which it contains is present in

too small an amount to make possible its efficient transformation into body proteins, may be greatly enhanced in growth-promoting value by the addition of the missing amino-acid or acids. This has been illustrated in the case of gelatin and for the meat digests prepared by Abderhalden (1913). It will be readily understood that when a series of purified proteins was fed to young animals, the remainder of the diet consisting of 28 per cent. of "protein-free milk," together with non-nitrogenous nutrients similar to the following:

	Per Cent.
Protein	18.0
"Protein-free milk"	28.2
Starch	20.8
Agar-agar	5.0
Fat	28.0

the amino-acids furnished by the "protein-free milk" may have improved the quality of some or all of the pure proteins. This would change the experimental results so as to make it unsafe to draw conclusions concerning their relative nutritive values when fed without such an amino-acid supplement.

Osborne and Mendel held the view that the amount of protein or its equivalent, resulting from the digestion of milk protein or directly secreted by the mammary gland, which was contained in their "protein-free milk," was too small to be a factor of significance in their experiments. They finally abandoned the use of the product in 1917, at which time they adopted essentially the method of study which has been described.

Nitrogen Compounds in "Protein-free Milk."—Some indication of the nutritive value of the nitrogenous compounds of "protein-free milk" is afforded by experiments carried out by McCollum and Davis (1915). They showed that young rats slowly lose weight when confined to a diet containing 1.5 per cent. of protein derived entirely from skim milk powder. They maintained their weights when the amount of milk proteins was increased to 3 per cent. of the food mixture. On the other hand, 1.5 per cent. of milk protein supplemented with nitrogen equivalent to 1.5 per cent. of protein derived from "protein-free milk" served to maintain rats approximately as well as did 3 per cent. from milk powder. They drew the conclusion that the nitrogen in "protein-free milk" has approximately the same nutritive value as that of milk proteins. This would not necessarily be

true when it serves as the sole source of nitrogen, but only when combined with the proteins of milk in equivalent amount.

Kennedy (1919) states that amino-nitrogen determinations before and after hydrolysis and the nitrogen distribution indicate that either unprecipitated protein or peptides of considerable size are present in "protein-free milk."

Sure (1920) found that "protein-free milk" contains 0.2 per cent. of sulphur, the greater part in organic form. His results suggest that "protein-free milk" contains either cystine or organically bound sulphur which the organism can transform into cystine. "Protein-free milk" gave a qualitative test for tyrosine.

Sure (1923) investigated the rôle of "protein-free milk" as a supplement to the total proteins in milk when casein and lactalbumin were introduced into the ration in relatively the same proportions in which they occur in milk. Lactalbumin had no supplemental value to the casein when the total proteins of milk were fed at 10 per cent. of the diet. When 28.3 per cent. of "protein-free milk" replaced 4 per cent. of salt mixture and 24 per cent. of dextrin, it showed considerable supplementary value to the total proteins of milk. Four-tenths per cent. of cystine behaved similarly; so the supplementary value of "protein-free milk" must be credited to amino-acids which it carries rather than to the salt mixture.

Lysine as Limiting Factor in Nutritive Value of Proteins.—Osborne and Mendel (1912) reported an experiment which they interpreted as demonstrating that the processes of maintenance are of a lower order than those of growth, and that repair of tissue can be effected without the amino-acid lysine, whereas this complex is indispensable for growth. There were two confusing factors in this experiment, viz., the "protein-free milk" nitrogen, and the erroneous belief that the protein, gliadin, contained but little lysine. They restricted a female rat for 178 days to a diet containing gliadin and "protein-free milk." It became pregnant and produced four young, which it suckled to the age of 23 days. This was presented as evidence that maintenance of the mother, the development of young in the uterus, and the formation of milk to nourish the young, are possible on a diet lacking lysine and incapable of supporting growth. Later studies on the content of lysine in gliadin show that no differentiation can be made of the nutritive needs of an animal during growth as contrasted with an adult drawing sustenance for repro-

duction and the rearing of young. This mother was being nourished on the lowest intake of lysine possible for her requirements under the experimental conditions. Hart, Nelson and Pitz (1918) found with rats that the mammary gland has not the capacity to synthesize lysine, and that it is not dispensable for maintenance.

Osborne had determined the amounts of lysine in numerous proteins and had found it to be 0.16 per cent. in gliadin. This is very small compared with the yields from many other proteins, notably those of animal tissues. This is illustrated by the following table (Osborne and Mendel, 1914):

LYSINE IN PROTEINS

	Per Cent.		Per Cent.
Lactalbumin	8.10	Glutelin (maize)	2.93
Halibut muscle	7.45	Glutenin (wheat)	1.92
Ox muscle	7.59	Edestin (hemp seed)	1.65
Casein (cow's milk)	7.61	Amandin (almond)	0.72
Vitellin (egg milk)	4.81	Gliadin (wheat)	0.16
Albumin (hen's egg)	3.76	Hordein (barley)	0.00
Legumin (pea)	4.98	Zein (maize)	0.00
Phaseolin (kidney bean)	4.58		

In another experiment they restricted young rats to a diet consisting of gliadin 18.0, "protein-free milk" 28.2, starch 20.8, agar-agar 5.0, and lard 28.0 per cent. The animals were able to maintain their weight for 500 days, but were unable to grow to any appreciable extent. When lysine was added to the diet to the extent of 3 per cent. of the protein content, normal development ensued.

At the time these experiments were conducted with gliadin it was assumed that this protein contained but 0.16 per cent. of lysine. Osborne, Van Slyke, Leavenworth and Vinograd (1915) made new analyses of gliadin by a more satisfactory method and found that it contains about 1.21 per cent. of lysine, or about seven times more than it was earlier assumed to contain. With this knowledge, and with the fact made evident that the uncharacterized nitrogen of "protein-free milk" in the quantities used in their experiments amounts to about half the maintenance requirement of a young animal, there is no reason to postulate a dissimilarity in the amino-acid requirements for maintenance as contrasted with growth.

Methods for Comparing Nutritive Values of Proteins.—

One method which Osborne and Mendel employed to illustrate the striking differences in the values of the proteins from different sources for the support of growth, was that of feeding a series of groups of animals on a diet of constant composition, but with an isolated protein, as a component in the diet of each experimental group. The amount of growth or of loss in weight of the animals during the first 30 days on the experimental diets was made the basis of comparison. The diet contained the following substances:

	Per Cent.
Purified protein	18.0
"Protein-free milk"	28.2
Starch	20.8
Agar-agar	5.0
Fat	28.0

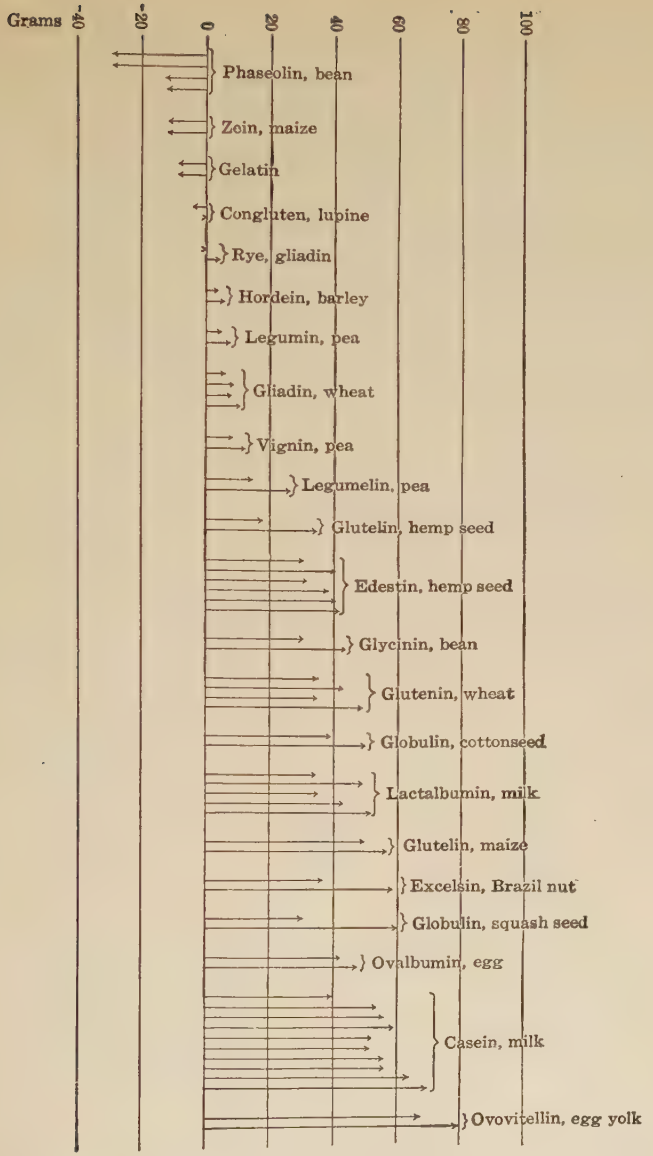
The results obtained with a series of proteins are shown in graphic form in the diagram on page 53.

An inspection of this table shows that rats restricted to bean protein lost weight rapidly. The ones fed zein and those fed gelatin lost weight, but not so rapidly. Conglutin, from lupine, and gliadin, from rye, served to maintain weight without growth, and little growth was secured with hordein of barley, or legumin of pea. All the other proteins listed were capable of promoting growth when fed with the other substances in the diet.

A second method which they employed for comparing the biological values of different proteins for growth in the rat, was to observe the rapidity of growth on diets, of the type described above, which contained but a single protein. The character of the protein varied in different experiments (1914).

They fed young rats diets, the protein contents of which were below the amount necessary for growth. The animals remained stunted for long periods. When the amount of protein in the diets was increased, they were able to resume growth at rates faster than rats ordinarily grow on a "normal" diet. From the results the deduction was drawn that interruption of growth does not result in the loss of the capacity to grow (1914 and 1915).

This generalization is too sweeping. It is true that rats which are stunted by means of protein starvation retain for long periods their capacity to resume growth. The form of the grown animal is closely similar to that of rats whose growth to adult size has been uninterrupted. McCollum and Simmonds (unpublished data) have found that young rats kept upon diets, the



The above table is slightly modified from a publication by Osborne and Mendel, Zeitschr. f. physiol. Chem., 1912, lxxx, 307.

inorganic content of which was unsatisfactory, develop abnormal forms, and become deformed. Any nutritive regimen which will induce rickets will markedly interfere with subsequent growth.

Animals whose growth has been suspended by depriving them of either vitamin A or B, are able to resume growth upon the inclusion of the missing substance in the diet. It is impossible, however, to carry animals on such diets for long periods.

A third method which Osborne and Mendel used was to carefully establish the amount of increase in body weight which was possible as a result of the consumption of a definite amount of protein. The rest of the food mixture remained essentially constant throughout the series of experiments (1915). As an illustration of the results secured by the latter method the following table is representative:

TABLE II

Rat	*Diet*	*Initial body weight*	*Gain*		*Intake of*	
					Total food	*Protein*
		grams	grams	days	grams	grams
2115	9% lactalbumin	65	99	80	589	43.7
2123	" "	43	89	80	395	29.3
2207	" "	59	91	80	575	42.6
2210	" "	60	93	80	560	41.5
2051	9% casein (cow's milk)	42	59	80	435	35.2
2110	9% edestin (hemp seed)	52	60	80	465	38.9
2044	4½% lactalbumin (milk)	42	44	80	371	13.8
2049	4½% lactalbumin	41	54	80	454	16.8
2118	4½% casein	59	–3	80	366	14.8
2113	4½% edestin	93	14	80	459	19.2
2114	4½% casein	65	29	80	464	19.4

It will be observed that rats which ate similar amounts of food grew much faster on 9 per cent of lactalbumin than on casein or edestin. Similar differences were observed on the lower protein intake. The proteins fed in these experiments were in all cases supplemented by nitrogen of "protein-free milk," so the results do not represent actual differences in values of the proteins named.

Uncertainty Concerning Biological Value of Lactalbumin. —The more recent experimental studies of lactalbumin make it impossible to interpret the results obtained with this protein. Osborne and Mendel (1917) secured growth with lactalbumin

when the diet contained "protein-free milk" as a source of vitamin B, but little or no growth when yeast furnished the vitamin. McCollum, Simmonds and Parsons (1919) were unable to secure growth with a diet in which lactalbumin was the only protein.

Sure (1920) also stated, "lactalbumin when fed at 12 and 18 per cent. of a ration, carrying 2 per cent. of the total protein in the form of nitrogen of unknown source in an alcoholic extract of wheat embryo to furnish the water-soluble vitamin, was found to be inadequate for growth. When fortified with a weight of cystine equal to 1 per cent. of that of the total protein, lactalbumin fed at 12 to 18 per cent. level was found to be of excellent nutritive value. Cystine, then, is the primary limiting factor in that protein." However, Osborne and Mendel (1924) have more recently reported good growth with diets containing only lactalbumin as a source of protein, with a yeast fraction fed as the source of vitamin B. They attribute the different results of the several investigators to a difference in the amount of vitamin B in the diets employed (1917).

Amino-acid Requirements in Maintenance and in Growth.—Soon after the studies which led Osborne and Mendel (1912) to conclude that the amino-acid lysine is not necessary for maintenance but is indispensable for growth, Osborne and coworkers (1915) made an analysis of the principal proteins of the endosperm of wheat, maize, and rice kernels. They found them to yield 1.58, 0.97, and 4.26 per cent., respectively, of lysine. They correlated these results with those which Thomas (1909) secured in brief experiments upon himself and stated, "It is rather striking that the figures for the utilizability of these proteins correspond so closely with the lysine which they yield." The three day type of digestion trial employed by Thomas, in which the factor of growth is not concerned, cannot be regarded as affording data comparable with the standards required in present-day experimental work.

Nollau (1915) and Buckner, Nollau and Kastle (1916) interpreted Osborne and Mendel's data as showing that, "lysine is primarily responsible for the stimulation of growth." They apparently regarded the planning of farm rations to insure a sufficient amount of this amino-acid as a practical agricultural problem. They fed chicks such complex mixtures as wheat, wheat bran, sunflower seed, hemp seed and skim milk, and contrasted the growth and well-being of these chicks with others restricted to barley, rice, hominy, oats, and gluten flour. The

former were well nourished and grew normally, while the latter were stunted. Buckner, Nollau and Kastle interpreted their results as due to "differences in the amino-acid content of the two rations and in all probability to differences in the lysine content."

It will be observed that the poor ration was limited to cereal grains and their by-products. One of the most important generalizations that can be made as the result of modern nutrition investigations is that it is impossible to secure a diet composed entirely of seeds which will promote good nutrition over any considerable period. The diet in question was poor in lysine and its mineral content was deficient in certain elements, notably calcium, phosphorus, sodium, and chlorine. Growth would not have been possible in their chicks, even if the proteins had been of better quality. Furthermore, this diet was very deficient in vitamin A. The good diet on the other hand was composed in great part of whole seeds, the germ of which improved the quality of the mixture of vegetable components. The bran was important as a source of mineral salts. When, in addition to these differences between the two diets, the presence of skim milk in the successful one is considered, it is apparent that the diets were in no sense comparable in regard to factors other than lysine. A liberal addition to their poor diet of lysine, or of all the essential digestion products of protein, would not have changed the outcome of their experiment.

In a later study, Osborne and Mendel (1916) employed maize gluten, which contains about 1.0 per cent. of lysine, supplemented with "protein-free milk" sufficient to furnish 1.33 per cent. of the total nitrogen of the food mixture, as the source of protein for growing chicks. They compared the growth obtained with this protein mixture with that obtained with rations similar in their make-up, except that the maize gluten nitrogen and "protein-free milk" nitrogen were supplemented with lactalbumin or cotton-seed flour. The latter supplements are rich in lysine. The results were interpreted as confirming the conclusions of Buckner, Nollau and Kastle in respect to the effect of foods high and low in lysine on the growth of chicks.

From the standpoint of practical nutrition these experimental studies all tended to focus attention upon lysine as an amino-acid of more importance than other indispensable digestion products of proteins, because it is relatively less abundant in proteins of the cereal grains.

The view that the content of lysine is especially likely to be

low in vegetable proteins is reflected in the publications of Johns and his co-workers (1917, 1918), who emphasized the importance of discovering among the vegetable foods sources of proteins rich in the basic amino-acids, arginine, histidine, and lysine, especially lysine. This view is justified by the known peculiarities of the cereal proteins which form so important a source of protein in the nutrition of man and animal. Osborne and Mendel have shown that the cereal proteins are especially deficient in this group of protein-building units.

No generalization can be made regarding the cause of low

FIG. 5.—The rations of these two rats had the same composition as shown by chemical analysis. They differed only in the source of the protein which they contained. The rat on the right grew up on a mixture of proteins from the corn kernel and wheat gluten; that on the left a mixture of corn proteins and gelatin. The difference in size, and remarkable difference in appearance is solely the result of the difference in the quality of the proteins in the two diets. Corn proteins and gelatin do not supplement each other's amino-acid deficiencies. These animals were the same age when photographed, and had been confined for the same number of days to the experimental diets.

biological value of individual proteins. While lysine is the limiting factor in some proteins, it appears not to be more frequently so than certain other amino-acids. While it is logical on theoretical grounds to combine a food which is known from the results of chemical studies to be low in lysine, or any other essential amino-acid with another food especially rich in such compound, it is not safe to place much confidence in the results of chemical analysis. This is illustrated by the deductions regarding the special importance of lysine in foods. McCollum, Simmonds and Pitz (1916) showed that poor as are the proteins of maize kernel in lysine, this is not the amino-acid present in smallest amount in proportion to the amount required for the formation of new tissues in growth. It is not the limiting factor in determining the value of the maize kernel proteins. A food

mixture was used which consisted of a single grain so supplemented with inorganic salts and butter fat as to be satisfactory for the promotion of growth, except that the protein was too low in amount to promote growth at the normal rate. In all cases the diets were shown to be made adequate for growth by the addition of a purified protein alone, when that protein was casein. This is a complete protein for nutrition. Rations containing wheat, maize or oat kernel were fed to young rats, and the same rations supplemented with gelatin or zein were fed to other groups. Zein, the most abundant protein in maize, is entirely lacking in tryptophane and lysine, whereas gelatin contains about 6 per cent. of lysine.

Obviously, if lysine were the limiting amino-acid in each of these grains, the addition of gelatin should enhance the value of the proteins in all cases and the nutrition of the animals should be correspondingly improved. The addition of zein, on the other hand, should not lead to improvement. These experiments show that zein did not supplement the proteins of the wheat or maize kernels, but did improve the proteins of the oat kernel. Later investigations have caused McCollum and Simmonds (unpublished data) to question these conclusions so far as the oat kernel is concerned. Lysine may possibly be the limiting factor in the oat kernel. Hogan (1916) found that the addition of lysine and tryptophane to maize did not enhance its nutritive value for growth of swine.

Gelatin supplements the proteins of both wheat and oat kernel in an efficient manner, but not maize kernel proteins. The improvement of diets containing maize gluten, which Osborne and Mendel (1917) observed to follow the introduction of lactalbumin or cotton-seed proteins, was apparently not the result of the addition of lysine.

Some further deductions are possible from these data. They support the view, but do not establish it, that lysine is the limiting factor in oat and wheat proteins. Gelatin contains no tryptophane or tyrosine and but a trace of cystine; this suggests that none of these three amino-acids are the ones present in minimal amounts in wheat or oat proteins (see Fig. 5).

Basis of Nutritive Value of Proteins.—The work reviewed establishes that the nutritive value of proteins is determined by their yields of the eighteen or more amino-acids formed on digestion. The more nearly these proportions correspond to the content of amino-acids in the tissues of the growing animal, the

more effectively can food proteins be transformed into body proteins.

There is no conclusive evidence that the process of maintenance, or the repair of tissue waste, are of a lower order than are the processes of growth, and that the needs of the former are met by a list of amino-acids which would be lacking in certain ones which are essential for the latter. The high utilization of protein when fed sparingly in a diet otherwise complete, suggests that there is such a difference between maintenance and growth. It has not been possible to demonstrate that through the agency of the mammary gland, amino-acids (e.g., lysine) can be synthesized for the nutrition of the young. The evidence all points to the conclusion that the transformation of food protein into milk protein is governed by the same laws which apply to growth.

Protein is but a single factor in nutrition. The content of essential mineral elements in the diet, and of the several unidentified factors, the absence of which leads in each case to the development of a pathological condition, are of equal importance from the standpoint of well-being. The discovery of the means of making the nice adjustments in a quantitative way among all the factors best adapted to promote optimum development, and of the means of preserving the harmonious rhythm of the metabolic processes, is the end sought by students of nutrition.

CHAPTER V

SOME PROBLEMS OF PROTEIN NUTRITION

Protein as a Structural Material.—The most prominent component of the body tissues, with the exception of the skeleton, is protein. It is the basis of the muscles and glands, and overshadows all other organic compounds in relative abundance. Liebig believed that a muscle does mechanical work at the expense of energy stored in it in the form of protein. He believed that energy came from the breakdown of the complex proteins into simpler products analogous to the change of complex substances of wood into the products of combustion. He believed that the energy set free was capable of appearing in part in the form of work.

If this view were correct, the amount of protein broken down daily should be profoundly influenced by the amount of work performed. Modern investigations have established, however, that work is ordinarily performed at the expense of the energy of carbohydrates such as starch and sugars, or from fat. This was first shown by Pettenkofer and Voit in 1866. These investigators observed that a fasting man showed no increased protein destruction as measured by the amount of nitrogen eliminated in the form of waste end-products in the urine. Instead of prodigally wasting his muscle substance (protein) he burned fat as a source of energy. Scott and Hastings (1920) have shown that *following severe work* there is an increased elimination of nitrogen.

For many years physiologists assumed that the tissues of the human or animal organism were not capable of synthetic transformations so as to give rise to the formation of complex organic compounds from simple ones. The building up of organic substances from inorganic compounds was believed to occur only in plants during growth. The logical inference was that the wear quota of the protein-rich tissues must be replaced by new proteins from the food. Early observations on fasting men and animals established the fact that even during periods of absti-

nence from food, tissue waste still went on, and in the absence of protein in the diet the muscles and other tissues were used up. During a subsequent period of feeding the lost muscle substance may be completely restored. Carl Voit (1872) determined the amount of protein in the form of meat which would suffice to prevent a nitrogen (protein) deficit in dogs. He did not actually determine the minimum amount of protein necessary to accomplish this. He showed that with moderately large intakes of meat protein, the nitrogen which appeared in the urine as waste products of metabolism, plus that eliminated in the feces, was equal to that ingested in the food. In other words, the body was in nitrogen (or protein) equilibrium.

Gelatin an Incomplete Protein.—Gelatin, which results from incipient hydrolysis of connective tissue and the organic matrix of bone, is a protein of peculiar nature. It was one of the first to be studied by chemists. Gelatin and the casein of milk were among the first proteins to be separated in a state of approximate purity. Voit attempted to substitute gelatin for a part or all of the meat protein in the diets of his dogs and discovered that gelatin as the sole source of nitrogen could not prevent loss of body protein. There was something lacking in its makeup which made it an incomplete food. Gelatin therefore became a substance of unique interest to physiological chemists.

Örum (1879) and Munk (1894) confirmed the earlier observations that a part of the protein could be replaced by gelatin, but that when it forms the sole source of nitrogen there is always a deficit.

Kossel postulated the chemical nature of the protein molecule to be a chainlike structure consisting of a number of amino-acids linked together. He believed the amino-group of one to be united to the carboxyl group of its neighbor in the chain through abstraction of the elements of water, and that digestion consisted of the addition of the elements of water and the dissolution of the union. The correctness of this view was proven through synthesis by Curtius and by E. Fischer.

Objectives of Research on Protein Nutrition.—Researches in the field of protein nutrition during the last 25 years have had as their main objectives the solution of the following problems: (1) The digestibility of the proteins; (2) the number and nature of the amino-acids contained in proteins of plant and of animal origin; (3) the amounts of each of these yielded by different proteins; (4) the extent of the digestive decomposition of

proteins in the alimentary tract; (5) the ability or inability of the tissues to synthesize the various digestion products of protein; (6) the immediate disposition of the products of protein digestion after absorption; (7) the chemical changes which take place during the catabolism or destruction of the amino-acids in the body; (8) the capacity of the body to synthesize protein when the requisite amino-acids are supplied in the food; (9) the determination of the relative efficiency possible in the transformation for purposes of growth of (a) individual proteins and of (b) the mixture of proteins in individual food-stuffs into body proteins; (10) a comparison of the number and kinds of amino-acids required in the process of repair of tissue waste, which is an essential result of life activities with the requirements for the formation of new tissues during growth; (11) the physiological effects of nutrition with protein intake at different planes, or the discovery of the amount of protein from stated sources which serves to maintain the body in optimum nutritive condition.

It is not within the scope of this book to discuss all phases of this subject. Only the last four problems will be considered. This is desirable because the literature relating to research along these lines is especially in need of being summarized and interpreted.

After the composition of the proteins became known, physiological chemists interested themselves in trying to demonstrate which, if any, of the 18 or more amino-acids or digestion products of proteins are indispensable in the diet. Henrique and Hansen (1905) published studies which led them to believe that the three diamino-acids, arginine, histidine and lysine, are not necessary in the nutrition of the rat. They kept a nitrogen balance on rats whose diets were believed to be lacking in these amino-acids. They doubtless failed to remove completely from their protein the amino-acids in question.

Kaufmann (1905) conducted the first experiment of a type which has been greatly extended by others. He fed dogs on diets in which gelatin was supplemented with tyrosine, cystine, and tryptophane. It was found possible with this mixture to replace fully the protein lost through metabolism. These results he confirmed by experiments on himself. In 1905, when his investigations were made, no one suspected the necessity of including vitamins in the diet. His experiments were so short that these omissions in the diet did not seriously interfere with the results.

Researches of Folin on Protein Metabolism.—Folin (1905) published three remarkable papers. He showed that the destruction of the greater part of the protein digested and absorbed, and its immediate conversion into waste products which constitute the normal components of the urine, differs markedly from the disposition of protein involved in the normal functioning of the tissues. The end-product of the first type is principally urea, while creatinine and uric acid are prominent components of the latter. When a diet free from protein or other nitrogenous compounds is taken in amount sufficient to cover the energy requirements of the body, the urine will, after a few days, contain about 60 per cent. of its total nitrogen in the form of urea, 18 per cent. as creatinine, 7.0 per cent. as ammonia, and 3.0 per cent. as uric acid. The remainder, which will amount to about 12-14 per cent. of the total, is in forms which are in great measure uncharacterized, and have not been estimated individually. A urine having this composition represents what Folin termed endogenous metabolism. He distinguished it from exogenous metabolism, which is concerned with the destruction of food protein that has never become a part of the living protoplasm. The magnitude of the endogenous process is surprisingly constant, whereas that of the exogenous type is very variable, and is determined by the consumption of protein. On ordinary planes of nutrition, exogenous metabolism or destruction of food is superimposed upon the endogenous or tissue waste type and the composition of the urine gives a composite picture.

Willcock and Hopkins' Studies on Zein.—Willcock and Hopkins (1906) made a study of the incomplete protein, zein, which was comparable with Kaufmann's studies with gelatin. Zein, which is the principal protein of maize, is entirely lacking in both lysine and tryptophane. These investigators fed young mice purified food substances in which zein was the sole source of protein (amino-acids), and observed that they died in less than 14 days in a state of torpor. When tryptophane was added to the food they lived about twice as long and remained lively until shortly before death. The addition of tyrosine did not improve the diet. They suggested that tryptophane may serve a two-fold function. Since it is an essential structural unit of the protein molecules they thought it might be a precursor of a hormone or regulator of metabolism which certain tissues elaborate.

It has been abundantly demonstrated that the simplest of the

amino-acids, glycocoll, is readily synthesized by the animal tissues (Magnus Levy, 1907). Casein contains no glycocoll but is a complete protein for growth. The tissues of the higher animals are probably capable of synthesizing certain amino-acids, but with the exception of glycocoll the ones which can be so synthesized are unknown.

Michaud (1909) sought to verify the theory that the more similar the food proteins to those of the tissues which they are destined to form in the body, the higher would be their biological value. He allowed dogs to fast for a time to reduce their nitrogen output to the minimum of tissue destruction. He then fed a non-nitrogenous diet supplemented with various amounts of proteins from different sources. His findings indicate that the proteins of the same species of animal are superior in dietary value to proteins obtained from another species or from plants. Dog muscle and dog serum proteins were of higher nutritive worth for the dog than muscle or serum proteins from the horse. A mixture of dog tissues (muscle, blood and glandular organs) was also superior to any of the other proteins studied. Gliadin and edestin, proteins from wheat and hempseed respectively, were of the lowest value. His diets were not comparable in quality with respect to factors other than protein. With the exception of those in which the glandular organs were included, the diets were very deficient in vitamins, and all were poor in their mineral content. His interpretation of his results was fallacious.

Protein Requirements in Maintenance and in Growth.—McCollum (1911) attempted to utilize Folin's discovery as a means of finding whether an amino-acid supply which is inadequate for the support of growth might be satisfactory for maintenance. A farm pig was given a nitrogen-free diet of starch, salts and water until the exogenous metabolism disappeared, as shown by the composition of the urine. In a subsequent period a definite amount of an incomplete protein such as gelatin or zein was added to the diet. From the increase in the nitrogen output in the urine and from the change in the forms in which it appeared, it was believed possible to decide to what extent the amino-acids of the incomplete protein were utilized.

Pigs readily ate sufficient of the nitrogen-free diet to cover their energy requirements. They could easily be brought into a condition in which they exhibited only the endogenous type of metabolism. When a known amount of nitrogen in the form of urea was added to the diet of pigs in this condition, it was all

recovered in the urine *in addition to the amount derived from endogenous sources.* The percentage composition of the urine with respect to creatinine and urea showed the expected alterations, the former representing less and the latter more of the total nitrogen of the urine.

When gelatin or zein was administered instead of urea in quantities sufficient to supply just the amount of nitrogen derived from endogenous metabolism, *the nitrogen from these "incomplete" proteins was only in part excreted in the urine in addition to the amount derived from the endogenous sources.* The results showed that 60 per cent. of the nitrogen of gelatin was excreted and 40 per cent. retained for some purpose in the tissues, presumably for the partial repair of tissue waste. Under the same experimental conditions 73 per cent. of the nitrogen of zein was retained and 27 per cent. excreted in addition to the amount expected from endogenous sources. The same criticism now applies to these experiments as to those of Kaufmann (1905), but they appear to prove a usefulness of "incomplete" proteins for tissue regeneration whereas they cannot induce growth (page 55).

Body Protein Synthesized from Amino-acids.—Folin and Denis (1913), Van Slyke and Meyer (1913) and Davis and Whipple (1919) have established that protein is readily synthesized from amino-acids. In the intestine the protein is digested to amino-acids, and the identity of the food proteins is lost. In the process of growth, in the regeneration of liver tissue destroyed as the result of chloroform narcosis, or in the replacement of the complexes in the tissues lost through the disintegration of certain structures in normal metabolism, the amino-acids derived from food protein are used for the construction of new proteins of structures different from those of the food.

Feeding Experiments with Amino-acids.—Ackroyd and Hopkins (1916) fed mice the essential non-nitrogenous nutrients supplemented with simple mixtures of purified amino-acids. They concluded that if arginine is present in the diet, histidine may be omitted, and vice-versa. A similar relationship they found between tyrosine and phenylalanine, the structural formulas of which are closely similar. These were the first experiments where it was believed it was demonstrated that one amino-acid may be converted into another.

Chemical Composition of Protein.—Kossel, Fischer and Osborne made it clear that there existed very pronounced differences in the composition of the proteins from different sources.

The proteins were prepared in a state of relative purity, were digested in the laboratory by means of acids, and were analyzed by the methods of Fischer and of Kossel. Certain of the eighteen digestion products, the amino-acids, were determined quantitatively so far as the methods would permit. Although the technique was never perfected so as to give results approximately quantitative, except in the case of less than a third of the amino-acids known, it was shown in the case of these few that there were great variations in the proportions among them in the mixtures obtained from proteins from different sources. Thus, the proteins of the muscle tissues of several species of animals yield between 12 and 14 per cent. of glutamic acid, one of the products of hydrolysis obtained from most proteins. This amino-acid is present in the two principal proteins of the wheat kernel to the extent of about 40 per cent. These two proteins together make about 85 per cent. of the total protein of the wheat kernel. Other equally great differences were shown to exist in the composition of proteins of common foods, and those of the tissue proteins of animals.

The problem which the animal meets in its protein nutrition may be illustrated by comparing the digestion products of the protein molecule to the letters of the alphabet. The proteins of the food and of the tissues which are unlike each other may be regarded as made up of the same letters arranged in different orders and present in different proportions. In growth, the food proteins which the animal takes are taken apart into simple compounds, the amino-acids, which are absorbed and put together in new order, and in new proportions to form the tissue proteins.

If the muscle tissue of an animal be compared to a block of printer's type so arranged as to print the rhyme beginning "Jack Spratt could eat no fat, and his wife could eat no lean," let the proteins of which the muscle consists be represented by the individual words, and the protein digestion products by the letters of which the words consist. If now the animal should take food proteins which correspond to a block of type which would print the jingle beginning "Peter Piper picked a peck of pickled peppers," it is easy to understand that when the proteins of the food are resolved into their constituent letters, and an effort is made to form the body proteins of the new and different type from the letters supplied by the food, the transformation cannot be made. In "Jack Spratt could eat no fat and his wife could eat no lean," we need four of the letter t, but the food proteins

contain but one. The first line of the Jack Spratt rhyme, which represents the muscle proteins, contains but one p, whereas the food proteins expressed by the Peter Piper rhyme yield nine in the first line. The first line of the Jack Spratt rhyme contains the letters j and n, whereas the Peter Piper rhyme contains none, so that even with the entire stanza:

> Peter Piper picked a peck of pickled peppers,
> If Peter Piper picked a peck of pickled peppers,
> Where's the peck of pickled peppers
> That Peter Piper picked?

it is not possible to produce even the first line of the Jack Spratt rhyme, and in order that growth might become possible it would be necessary to take proteins of another character which would supply the missing letters.

Zein, of corn, is entirely lacking in three of the amino-acids or digestion products which are obtainable from most tissue proteins. In accordance with what we should expect on theoretical grounds, this protein is incapable of supporting growth, or of maintaining an animal in body weight when taken as the sole source of amino-acids. This illustration shows how we may have superior, good or inferior food proteins for the formation of body proteins in growth.

CHAPTER VI

PECULIARITIES OF COMPOSITION OF PROTEINS FROM VARIOUS SOURCES

Certain Amino-acids Indispensable for Nutrition.—In Chapter V an account was given of the experimental studies which established the fact that the nutritive value of a protein depends upon the number and amounts of the amino-acids it yields on digestion. Proteins such as gelatin, lacking in the amino-acids, tyrosine, cystine and tryptophane, or zein of maize, deficient in lysine, are incomplete for all physiological purposes, since they cannot supply all the "building stones" necessary for the construction of body proteins. The nutritive value of a protein or mixture of proteins depends upon the presence in its molecules of all the essential amino-acids, and upon the extent to which their proportions correspond to those existing in the body proteins into which they are to be transformed. One protein may supplement the deficiency of another. On these facts was based a criticism of certain of the experimental work designed to yield data showing in a quantitative way the comparative values of different purified proteins.

In order to illustrate further the differences in the constitution of individual proteins from different sources, a few tables are presented which summarize the best analytical data obtainable by chemical methods. Table III shows the composition of a series of animal and vegetable proteins and the sources of origin. The elements carbon, hydrogen, nitrogen, sulphur and oxygen, are designated by the symbols C, H, N, S and O respectively.

Elementary Composition of Proteins.—Interesting information to be gained from this table is the remarkable similarity of the proteins from various sources in respect to their content of carbon, hydrogen and oxygen, and their relatively wide differences in nitrogen and sulphur content. Ovalbumin of the hen's egg contains but 15.51 per cent. of nitrogen, whereas amandin from almond contains 19.32 per cent. Legumin from bean contains but 0.385 per cent. of sulphur, while serumalbumin from

TABLE III

Elementary Composition and Molecular Weights of Proteins*

PERCENTAGE COMPOSITIONS						FORMULA						
Source of Protein	*C*	*H*	*N*	*S*	*O*	*Name of Protein*	*C*	*H*	*N*	*S*	*O*	*Molecular weight*
						Plant Proteins						
1. Almond	51.30	6.90	19.32	0.429	22.05	1. Amandin	638	1030	206	2	206	14930
2. Pea	52.64	6.95	17.25	0.426	22.73	2. Vignin	660	1040	185	2	214	15038
3. Bean	51.72	6.95	18.04	0.385	22.90	3. Legumin	718	1158	214	2	238	16642
4. Maize	55.23	7.26	16.13	0.600	20.78	4. Zein	736	1161	184	3	208	15993
5. Soy bean	52.12	6.93	17.53	0.710	22.71	5. Glycinin	780	1248	206	4	255	17700
6. Barley	54.29	6.80	17.21	0.847	20.53	6. Hordein	675	1014	181	4	194	14880
7. Hempseed	51.50	7.02	18.69	0.88	21.91	7. Edestin	624	1021	193	4	199	14523
8. Bynin	55.03	6.67	16.26	0.84	21.20	8. Bynin	706	1026	180	4	204	15410
9. Wheat	52.72	6.86	17.66	1.027	21.73	9. Gliadin	685	1068	196	5	211	15568
10. Para nut	52.18	6.92	18.30	1.086	21.51	10. Excelsin	642	1018	192	5	198	14738
11. Wheat	53.02	6.84	16.80	1.280	22.06	11. Leukosin	663	1026	180	6	207	15006
						Animal Proteins						
12. Ox	54.98	7.20	16.89	0.42	20.51	12. Globin	700	1098	184	2	196	15274
13. Ox	52.68	6.83	16.91	1.10	22.48	13. Fibrin	645	1004	178	5	207	14708
14. Horse	52.71	7.01	15.85	1.11	23.32	14. Serumglobulin	628	1002	160	5	209	14310
15. Horse	52.93	6.90	16.66	1.25	22.26	15. Fibrinogen	679	1062	183	6	207	15276
16. Muscle	52.82	7.11	16.67	1.27	22.03	16. Myosin	660	1074	181	6	208	15048
17. Egg	52.75	7.10	15.51	1.616	23.02	17. Ovalbumin	696	1125	175	8	220	15703
18. Cow's milk	52.19	7.18	15.77	1.73	23.13	18. Lactalbumin	644	1064	166	8	214	14792
19. Horse	52.99	7.01	15.93	1.93	22.14	19. Serumalbumin	662	1051	171	9	207	14989
20. Man	52.25	6.65	15.88	2.25	22.95	20. Serumalbumin	684	1045	178	11	225	15697
21. Horse	54.64	7.09	17.38	0.39	20.16	21. Oxyhaemoglobin	758	1181	207	2	210	16655
22. Man	54.57	7.11	16.38	0.568	21.04	22. Oxyhaemoglobin	758	1185	195	3	219	16667
23. Cow's milk	53 13	7.06	15.78	0.800	22.37	23. Casein	708	1130	180	4	224	15982
24. Egg yolk	51.56	7.12	16.23	1.028	23.24	24. Ovovitellin	671	1112	182	5	227	15628

*Osborne, T. B. Zeit. f. analyt. Chem., xli, 25, 1902.

human blood contains 2.25 per cent. of this element, or nearly six times as much for equal weights.

The Hausmann Method of Analysis of Proteins.—Table IV shows the constitution of different proteins as revealed by the Hausmann method of analysis. There is in every protein a complex which yields ammonia when boiled for a few hours with moderately strong mineral acids. In the table the per cent. of the total nitrogen convertible into ammonia is tabulated in the column under NH_3-N. There are three diamino-acids derived from proteins; these are differentiated from all the others by their basic character,—that is, they have an alkaline reaction. They can be precipitated together by means of phosphotungstic acid, and thus separated from all the other constituents of the protein molecule. The remaining amino-acids formed on digestion are collectively designated as mono-amino acids. When proteins are heated with acids as is necessary in the method under discussion, there is always formed a certain amount of black, insoluble nitrogenous substance called humin. It has been shown that most of this comes from the decomposition of the amino-acid, tryptophane. Some proteins (e.g., zein) are entirely lacking in tryptophane. Under "Total N" is given the per cent. of nitrogen in the samples analyzed.

An inspection of this table shows that the amount of nitrogen in the protein molecule which is convertible into ammonia under the conditions of analysis varies from 6.46 per cent. in legumelin of pea or bean to 23.78 per cent. in gliadin of wheat. In contrast with proteins of animal origin, it will be noted that many proteins of vegetable origin contain two to four times the amount of nitrogen in the ammonia-yielding complex. Data of this kind as early as 1905 showed that the proteins differ to a great extent in their constitution, and that food proteins are in most cases quite unlike the proteins which compose the muscles and organs of the body.

The method of analysis by means of which the data in Table IV were secured is not a very searching one, and fails to yield the detailed information which is desired for physiological studies. For this purpose the differences in the yields of the diamino-acids (bases) in different proteins do not, except in a few instances, contrast markedly with each other. In the case of glutenin, gliadin, hordein and zein, the principal proteins of wheat, barley, and maize kernels, the content of basic amino-acids falls below that present in any animal proteins. From

TABLE IV

DISTRIBUTION OF NITROGEN IN PROTEINS*

TOTAL N = 100

	NH_3 N	*Basic Amino Acids* N	*Mono-Amino Acids* N	*Humin* N	*Total* N
Vegetable Proteins:					
Conalbumin (egg white)	7.51	25.82	65.11	1.61	16.11
Phaseolin (bean)	10.74	24.50	62.85	1.79	16.20
Glycinin (pea).....	12.09	22.63	64.58	.68	17.45
Legumelin (pea, bean)..	6.46	23.06	68.12	2.36	16.09
Leucosin (wheat)	6.85	20.67	69.87	2.54	16.93
Glutenin (wheat).....	18.86	11.72	68.32	1.09	17.49
Gliadin (rye)	23.78	5.49	70.27	.79	17.66
Hordein (barley)	23.30	4.47	69.96	1.33	17.21
Zein (maize)	18.41	3.03	77.56	.99	16.13
Globulin (wheat)	7.72	37.14	53.39	1.52	18.39
Globulin (coca nut)....	7.35	32.79	59.09	.75	18.48
Globulin (squash seed)..	6.91	32.25	59.64	1.18	18.51
Globulin (cotton seed)..	10.30	30.63	59.06	....	18.64
Edestin (hemp seed)....	10.08	31.70	57.83	.64	18.64
Excelsin (para nut)....	8.87	31.47	59.94	.92	19.30
Corylin (hazel nut).....	11.57	30.26	56.31	.89	19.00
Amandin (almond).....	16.04	21.84	60.08	.89	19.00
Globulin (castor bean)..	10.45	30.08	58.66	.64	18.75
Corylin (walnut)	9.44	28.71	61.09	.79	18.84
Congluten (a) (lupine)..	11.84	29.05	58.00	1.00	17.90
Congluten (b) (lupine)..	14.55	28.17	56.56	.76	18.21
Legumin (pea)	9.40	28.82	60.27	.94	17.97
Globulin (flaxseed)	10.82	25.81	62.06	1.18	18.48
Vicilin (pea, bean).....	10.40	27.76	60.60	1.22	17.11
Vignin (pea)	11.07	25.39	62.66	1.45	17.25
Globulin (sunflower seed)	13.83	22.98	62.00	1.29	18.58
Stizolobin (Velvet bean)	10.51	27.96	60.06	1.36	16.40
Arachin (Peanut)	11.10	27.13	60.50	1.20	18.28
Animal Proteins:					
Vitellin (egg yolk).....	7.67	28.56	62.41	1.35	16.28
Casein (cow's milk).....	10.30	22.84	66.00	1.34	15.62
Ovalbumin (egg).....	8.64	21.27	68.21	1.87	15.51
Chicken muscle	7.45	29.98	59.85	2.43	16.09
Halibut muscle	6.70	30.18	60.73	2.37	16.40
Scallop muscle	6.33	26.51	64.81	2.34	17.05
Beef muscle	5.50	27.32	64.52	2.65	16.18

*Compiled from the papers of T. B. Osborne and of C. O. Johns.

Table V more is to be learned about this fraction of the protein molecule. There are three amino-acids in this group, called arginine, histidine, and lysine, which vary markedly in their occurrence in different proteins.

The Content of Arginine, Histidine and Lysine in Individual Proteins.—Table V contains analytical data obtained by the Kossel method for the estimation in proteins of arginine, histi-

TABLE V

CONTENT OF ARGININE, HISTIDINE AND LYSINE IN PROTEINS *

	Histidine	*Arginine*	*Lysine*
Vegetable Proteins:			
Globulin (Buckwheat)	0.88	23.67	8.61
Globulin (Squash seed)	2.42	14.44	1.99
Excelsin (Para nut)	2.50	14.29	1.64
Edestin (Hemp seed)	2.19	14.17	1.65
Globulin (Cotton seed)	3.46	13.51	2.06
Stizolobin (Velvet bean)	3.81	14.13	10.02
Globulin (Castor bean)	2.74	13.19	1.54
Amandin (Almond)	1.87	12.16	0.72
Legumin (Pea)	1.69	11.73	4.98
Legumin (Vetch)	2.94	11.06	3.70
Conglutin A (Lupine)	2.51	10.93	2.73
Vicilin (Pea)	2.17	8.91	5.40
Glycinin (Soy bean)	2.10	7.69	3.39
Vignin (Cow pea)	3.08	7.20	4.31
Glutelin (Maize)	3.00	7.06	2.93
Leucosin (Wheat)	2.83	5.94	2.75
Legumelin (Pea)	2.27	5.45	3.03
Legumelin (Soy bean)	2.04	5.35	4.91
Phaseolin (Kidney bean)	2.62	4.87	4.58
Glutenin (Wheat)	1.76	4.72	1.92
Gliadin (Wheat)	0.58	3.16	1.33
Gliadin (Rye)	0.39	2.22	...
Hordein (Barley)	1.28	2.16	...
Zein (Maize)	0.82	1.35	0.00
Animal Proteins:			
Ovovitellin (Egg yolk)	1.90	7.46	4.81
Beef muscle	2.66	7.47	7.59
Scallop adductor muscle	2.02	7.38	5.77
Chicken muscle	2.47	6.50	7.24
Halibut muscle	2.55	6.34	7.45
Conalbumin (Egg white)	2.17	5.07	6.43
Ovalbumin (Egg white)	1.71	4.91	3.76
Casein (Cow milk)	2.50	3.81	5.95
Lactalbumin	2.06	3.23	9.16

*This table is compiled from various sources, especially the papers of Osborne and of Johns.

dine and lysine. The histidine content of plant proteins varies much less than does that of either arginine or lysine. The extreme values for this constituent of the protein molecule range from 3.81 per cent. in stizolobin from the velvet bean, to 0.39 per cent. in gliadin from rye. The content of arginine in different proteins varies surprisingly. A globulin of buckwheat yields 23 per cent., whereas zein contains but 1.35 per cent. Among the vegetable proteins the yield of lysine is generally very low in comparison to that obtained from the proteins of animal tissues. The extreme limits of the yield of lysine in plant proteins range from 10.2 per cent. in the globulin of the velvet bean to complete absence in zein.

Comparison of the amounts of the three digestion products in vegetable and of animal proteins reveals a remarkable fact. While the amount of histidine is of the same order in the two classes, arginine is found in many seed proteins in quantities greatly in excess of any animal proteins. The only vegetable proteins yet studied which exceed muscle proteins in content of lysine are those of the velvet bean and buckwheat (Johns and co-workers, 1918, 1920).

It was the marked deficiency of most vegetable proteins in lysine, in contrast to animal proteins, that at one time led Osborne and Mendel and others to believe that this amino-acid was frequently the limiting factor which determined the nutritive value of proteins.

Variation in Glutamic Acid Content of Proteins.—Table VI shows the content of glutamic acid in a number of proteins of animal and vegetable origin. This amino-acid is a never-failing component of vegetable proteins and of animal proteins except the protamins in the spermatozoa of fish. This hydrolytic product is frequently much more abundant in vegetable proteins than in animal tissues or in egg or milk proteins.

From these data it is easy to understand why food proteins show great variations in nutritive value, for this depends upon the efficiency with which they can be transformed during growth, or the repair of tissue waste into tissue proteins. Since in human and in animal nutrition several proteins are always taken together, when vegetable or animal tissues are used as food, it is of great economic importance to discover what foods contain proteins which possess high supplementary values. Protein is the most expensive component of foods or feeding-stuffs, and agricultural economics can be greatly advanced by scientific discovery in this field. In human nutrition, economy, in ordinary

TABLE VI

PER CENT. OF GLUTAMIC ACID YIELDED BY PROTEINS*

Vegetable Proteins:	
Gliadin (Wheat)	42.98 per cent.
Gliadin (Rye)	37.80
Hordein (Barley)	43.19
Zein (Maize)	26.17
Leucosin (Wheat)	5.72
Glutenin (Wheat)	23.40
Amandin (Almond)	23.14
Edestin (Hemp seed)	14.00
Corylin (Hazel nut)	17.94
Phaseolin (Bean)	12.33
Vignin (Pea, bean)	16.30
Legumin (Vetch)	16.48
Glycinin (Soy bean)	19.46
Conglutin (Yellow lupine)	30.05
Animal Proteins:	
Casein (Cow milk)	10.77
Ovalbumin (Egg)	9.01
Conalbumin (Egg)	7.00
Vitellin (Egg yolk)	12.95
Serum albumin (Horse)	7.70
Serum globulin (Horse)	8.50
Halibut muscle	10.13
Chicken muscle	16.48
Scallop muscle	14.88
Dog tissues, entire body	14.00
Rabbit tissues, entire body	14.14
Hen tissues, entire body	12.02

* Compiled data from various sources, especially the papers of T. B. Osborne and E. Abderhalden.

times, is less important than well-being, but it would appear that it is no less important here that a protein supply of high biological value be provided in the diet. It seems logical to serve the needs of the body by providing it with the optimum amount of those proteins which can be utilized most effectively, rather than to require it to digest and assimilate an excessive amount of proteins of low value. In this connection it must be remembered that the moiety of amino-acids which cannot be properly matched with each other to form the necessary structures have to be destroyed and their degradation products eliminated as waste. This imposes an unnecessary burden on the organs concerned with the metabolism of the nitrogenous foods.

CHAPTER VII

SUPPLEMENTARY VALUES OF PROTEINS

Methods for the Study of Protein Values.—Since chemical methods for the analysis of proteins have not been sufficiently perfected to give a satisfactory knowledge of the differences in their composition, it is necessary to study this problem by means of experiments on animals. Several lines of procedure offer prospects of successful comparison of the relative biological values of mixtures of proteins. In order to study the effectiveness with which the proteins of one food supplement those of another, it is essential that diets be so planned that they are highly satisfactory with respect to all factors other than protein. The natures of the deficiencies of all the foods studied must first be well understood, so that any mixtures employed in experiments designed to reveal the biological values of combinations of proteins may be supplemented with respect to inorganic salts, the essential vitamins, etc. Then only will success or failure of growth, reproduction or other function in the animals, turn solely on the quality of the proteins of the diet. In Chapter IX the dietary properties of many of the more important of our foods are given. It is now appropriate to present the methods employed in the study of the protein values of combinations of these articles of diet. One procedure is to feed a series of groups of young animals a diet similarly constituted and deriving all of its protein from the same source, but with the per cent. of protein adjusted in the series from low to high levels. From the rate of growth of the animals an idea can be gained as to the least amount of protein from the sources studied which just suffices to maintain animals without growth and to induce growth at subnormal rates, or at the maximum rate.

Another method is to utilize female animals nursing litters of young. By adopting a standard size for the litter, and by observing the success with which the mothers induce growth in their young when the diet contains different percentages of protein but is otherwise of uniform composition, an approximately

quantitative comparison of the values of the proteins from different sources can be made.

Osborne and Mendel (1917) made attempts, in their studies with isolated proteins, to determine their relative values by observing the growth of animals which ate the same amount of food in the same number of days and gained the same amount in weight. The protein factor was the only variable. It is difficult to conduct experiments in this manner, so they (1919) attempted to develop another method by restricting the protein so that it became the factor which determined the rate of growth. They attempted to discover the concentration of protein in the diet which promoted the greatest gain of body weight

TABLE VII

Protein.	*Initial Body Weight. Grams.*	*Protein in Food. Per Cent*	*Intake Per Gram of Gain.*		*Gain Per Gram of Food.*	*Gain Per Gram of Protein. Grams.*
			Food. Grams.	*Protein. Grams.*		
Lactalbumin ..	62	16.2	3.3	0.52	0.31	1.93
	61	"	2.6	0.42	0.39	2.41
	73	"	3.4	0.54	0.30	1.86
	67	"	3.8	0.60	0.27	1.67
Average			3.3	0.52	0.32	1.97
Lactalbumin ..	71	10.3	3.6	0.37	0.28	2.74
	63	"	4.0	0.42	0.25	2.41
Average			3.8	0.40	0.27	2.58
Lactalbumin ..	64	7.9	4.0	0.31	0.25	3.18
	70	"	4.5	0.35	0.22	2.83
Average			4.3	0.33	0.24	3.01
Lactalbumin ..	66	6.2	6.3	0.39	0.16	2.58
	67	"	6.0	0.37	0.17	2.69
Average			6.2	0.38	0.17	2.64
Lactalbumin ..	63	4.9	8.6	0.42	0.12	2.40
	63	"	8.9	0.43	0.11	2.32
Average			8.8	0.43	0.12	2.36
Lactalbumin ..	68	3.3	18.7	0.62	0.05	1.63
	67	"	24.6	0.82	0.04	1.23
Average			21.7	0.72	0.05	1.43

relative to the protein ingested. To accomplish this they supplied the animals with food containing different percentages of the same protein. Using this method they secured data illustrated by Table VII.

In a similar study in which casein was the protein used, the protein intake was in most cases higher than in the lactalbumin experiments. For comparison the results are given in the table below in condensed form.

TABLE VIII

Protein	Casein
Per cent in food	17.4
Weights of experimental animals	58-76 grams
Intake per gram of gain	Food....3.1; 4.0; 3.0; 3.5; 3.9; 3.1; 3.5; 3.0; 3.4. Average, 3.4.
	Protein..0.54; 0.70; 0.53; 0.61; 0.68; 0.53; 0.61; 0.52; 0.59. Average, 0.59.
Protein	Casein
Per cent in food	14.7
Weights of experimental animals	64-67 grams
Intake per gram of gain	Food....4.1; 3.5; 3.0. Averge, 3.5.
	Protein..0.60; 0.51; 0.44. Average, 0.52.
Protein	Casein
Per cent in food	12.0
Weights of experimental animals	63-69 grams
Intake per gram of gain	Food....4.2; 3.5; 3.5. Average, 3.7.
	Protein..0.50; 0.42; 0.42. Average, 0.45.
Protein	Casein
Per cent in food	9.3
Weights of experimental animals	64-65 grams
Intake per gram of gain	Food....8.9; 6.4; 7.4. Average, 7.6.
	Protein..0.83; 0.50; 0.68. Average, 0.70.

Individual Variation in Growth of Rats.—Under the same experimental conditions and on the same diet the variation in gain of weight amounted to as much as 75 per cent., and the gains per gram of protein ingested varied as much as 10 to 30 per cent. in different animals. If we desire to learn the extent to which a food protein during growth can be converted into body proteins, it would be more logical to accept only the greatest gain observed in any animal, and to discard all other results, rather than to average the gains made by good and poor ones. Osborne and Mendel estimate that the gain of weight per gram of protein eaten during a period of eight weeks was for lactalbumin 2.34 and for casein 1.70. In three experiments with casein

fed at 12.0 per cent. of the food mixture, rats made an average gain of 2.25 gm. per gram of casein consumed. One attained a gain of 2.39 gm. and another of 1.99 gm. Their results are not very convincing as proof of the superiority of this method over other methods as a measure of the relative biological value of proteins.

Energy and Protein Used by Growing Animals.—The data of Osborne and Mendel discussed above have an interest quite apart from their worth as quantitative comparisons of the relative values of proteins. They support the view that "Economy of food can be effected only by supplying the young animal with as much as it will eat; economy of protein only by reducing the nutritive ratio below that at which the normal rate of growth can be maintained." This principle appears to be established, but it is quite another matter to determine whether or not economy of protein utilization obtained by the means just mentioned makes for physiological well-being. The studies of McCollum and Simmonds, to be described later, point to the desirability, for the long maintenance of vigor, of a liberal but not excessive supply of protein in the diet.

A procedure devised by McCollum and Simmonds (1919) for the study of the relative values of different proteins, or for the supplementary values of proteins from different sources, involves feeding a series of groups of animals on diets which are of uniformly excellent quality with respect to all factors except protein. The diets contain 9 per cent. of protein, which may be derived from a single food or from two or more sources. On such diets young rats are observed during the entire period and thereafter up to the time when pronounced developments of senile characters are noted. The fertility and infant mortality are observed. The behavior of the animals with respect to a tendency to destroy their new-born young when the protein supply is unsatisfactory, is also recorded. Nine per cent. of proteins of the highest biological value just suffices to promote good nutrition in rats over approximately the normal expectation of life. Proteins of lower value require to be fed at higher levels, otherwise growth is retarded or fertility decreased, and the span of life shortened. Sufficient vitamin E was present, since no diet contained less than 40 per cent. of natural foods.

McCollum and co-workers had conducted numerous experiments in feeding pigs diets containing different percentages of protein. The objective was a study of the relative values of purified

proteins and of the mixtures of proteins contained in individual natural foods, both for growth and for the replacement of nitrogen lost through tissue metabolism (1911 and 1913). In the early studies too little was known about supplementing factors other than protein. None of these results are very satisfactory since factors other than protein in the diets were not always suitably improved to make the results turn entirely on the quality of the proteins.

These studies showed, however, that all the amino-acids necessary for animal nutrition are contained in the proteins of wheat, maize, and oat kernels respectively. Certain ones are present in such limited amounts as to restrict the extent to which those more abundant can be utilized. For this reason these proteins are of relatively low value unless supplemented by proteins from other sources. With few exceptions the proteins of the cereal grains are of lower value than are those of milk and eggs, or of certain mixtures obtained by combining two or more of these seeds.

Quantitative Records of Food Consumption.—Many such studies on protein values were criticized by Osborne and Mendel because when rats were employed no records were kept to show the amounts of food consumed by each individual. They pointed to the great differences in amount of food eaten by different rats as evidence that there must be a correlation between food consumption and growth. They were able, by using large amounts of fat, to convert their food into a paste, which could be placed in a glass tube provided with a rod which acted as a plunger. Each rat was kept in an individual cage. From time to time food was expelled from the device into the feeding dish. They employed this device in their experiments, and furnished figures of food consumption.

McCollum and co-workers were unable to secure a quantitative record of food intake for their rats until about 1915, for they employed diets ground to fine powder. Their plan did not admit of the inclusion of large amounts of fat in their food mixtures, because this procedure diluted so much the mineral content, protein content, etc., of any natural food under investigation. Large amounts of fats make it impossible to feed sufficient amounts of grains and legume seeds to give an accurate picture of their dietary properties. It was therefore imperative that rats should be fed with diets low in fats. A device illustrated in Fig. 6 makes possible the quantitative feeding of powders. It is

possible to compare diets with respect to any component, e.g., protein, vitamin, or inorganic element, only when they have the same calorific value per gram, since animals eat for energy and not for protein or other element not adequately supplied. McCollum and Simmonds used only diets poor in fats, since this allowed greater freedom in modifying natural foods for study. With this device records of food intake are accurate to within 1 per cent. Since the animals are usually not kept in individual cages, but in groups of four to eight in one large cage, most of their records showed the food consumption of the group and not of an individual.

Good nutrition can be secured with diets planned as described, in which the proteins are derived solely from milk, kidney, or a mixture of certain seeds, notably of rye and flaxseed oil meal. So far this has not been achieved with rats on any diet containing less than 9 per cent. of protein from any source. McCollum, Simmonds and Parsons (1921) classed as excellent any protein or protein mixture which would support nearly optimum nutrition over periods approximating two-thirds or more of the normal span of life of the rat when fed in amount corresponding to 9 per cent. of the food mixture. Whereas, when Osborne and Mendel attempted to establish absolute values for the proteins, they sought only to compare any protein or mixture of proteins with some protein which, according to the criterion described, would be classed as excellent.

In all our protein studies, four to six rats were confined in a cage 24 x 24 x 20 inches, screened on all, or on three sides with wire cloth of one-quarter inch mesh. The food records always showed the amount consumed by the group. It is believed that a more detailed record of individual food consumption would add little value to the results or make their interpretation more significant. Exception must be made in experiments covering but a few days where the healing of rickets is being observed, since starvation causes confusing changes in bone structure. In rickets of severe grade little rats become unable to walk effectively, and so do not find their way to the food.

Other Methods for Comparing the Biological Values of Proteins.—Mitchell (1923-24) employs young rats of nearly 70 gm. weight. The animals are fed a complete ration, the protein being derived entirely from the source to be studied. The experiment covers five periods of ten days each. The urine and feces are collected quantitatively on the last six days of each period

and the six day composites are each analyzed for their nitrogen content. Records of daily food consumption are kept. The rats are weighed on the first day of the experiment and every ten days thereafter. The food is analyzed for nitrogen content. The biological value is defined as the per cent. of absorbed nitrogen retained in the body.

Analytical errors are involved relative to the collection of urine and feces and accumulate on three counts in nitrogen determinations in food, urine and feces, and also errors as to food consumption. Errors also occur because of individual variations in animals as regards their metabolic activity. A statistical

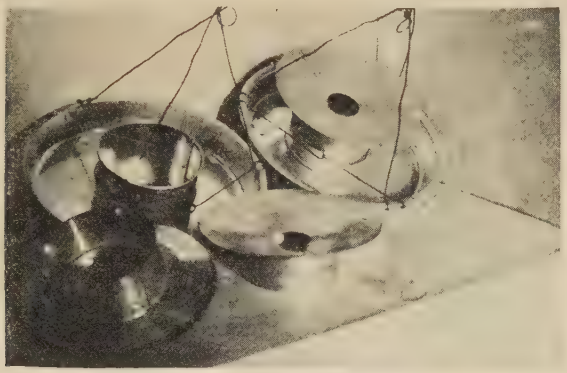


FIG. 6.—Type of feeding device described on page 79.

error is involved since Mitchell strikes an average of values which may in many cases be widely divergent. The calculations are rather involved so there is no direct relationship between the estimated biological value of a protein and any one of the factors involved. The method uses less time and fewer animals than other methods used in the past. The results check fairly well when averaged, notwithstanding the wide variations in any one set of experiments. Other workers have successfully duplicated his results.

When the proteins were fed at 5 per cent. of the diet the following biological values were obtained: Milk 93.4, casein 70.8, corn 72.0, oats 78.6, rice 86.1, potato 68.5, yeast 85.5. With proteins of corn, milk, oats, potatoes, navy bean and tankage at 10 per cent. levels the biological values estimated were respectively: 59.6, 84.7, 64.9, 66.7, 38.4 and 31.5.

McCollum (1911) emphasized that young swine fed diets containing a low percentage of protein exhibited higher apparent utilization of protein the nearer the maintenance level was approached. He suggested that the processes of repair of tissue waste are of a lower order than are those of tissue growth, and that a list of amino-acids which is incomplete for growth may be distinctly useful for replacing endogenous waste. All of the later studies on the nutritive values of proteins support the view that the highest apparent biological values are obtained when a protein is fed in amounts near the maintenance level. However, the true biological value of a protein is the extent to which it can be transformed into body protein during growth. It seems logical to expect that this value can be discovered only by experiments in which the level of protein intake is the maximum at which the animal is able to digest and assimilate, i.e., construct new tissue proteins.

A method employed by Kik and McCollum, and Shukers and McCollum (unpublished data) is much simpler than any method yet described. Nine rats as nearly 45 gm. in weight as possible, chosen from several litters of eight, are used in each experiment. Three of these are killed at the beginning of the experiment, the digestive tract washed clean; the live and empty weight of each rat is recorded. Each entire carcass is ground by passing repeatedly through a meat chopper, and then thoroughly mixed for sampling. The tissues are stored in glass-stoppered bottles in a refrigerator, and moisture, fat and ash are determined on each rat. The average nitrogen content of these controls is taken as that of the other experimental rats. Three of the remaining six are fed during 28 days a complete diet containing the protein being studied, and at the end of this period are killed and analyzed in the same manner as the controls. The remaining three are fed the same diet 56 days when they are also analyzed in like manner. Judgment as to the biological value of the proteins of the diet is based upon the nitrogen stored by the experimental animals during the feeding periods. This method requires more time than that of Mitchell but has fewer sources of error. With the feeding device illustrated on page 81 there need be no error in food consumption records, whereas in Mitchell's method some food is invariably spilled into the excreta and can scarcely be entirely separated. The nitrogen determinations check well, and the values employed in calculations represent the average of triplicate estimations.

The results obtained with several sources of proteins by the Mitchell and by the McCollum and Shukers methods, with rats of the same inbred stock, are shown in Table IX.*

TABLE IX

SUMMARY OF AVERAGE BIOLOGICAL VALUES AS DETERMINED BY THE THREE METHODS COMPARED

Lot	*Diet*	*Source of Protein*	*% Protein*	*% Nitrogen*	*Average Biological Values*		
					Osborne and Mendel	*McCollum*	*Mitchell*
1	4 4a	Ether extracted dried whole egg	8.25				99
1	3	Beefsteak	8.25				84
1 2	2 2	Whole wheat Whole wheat	8.25		1.180	25.0	82
5	7	Menhaden Meal	8.7	1.39	1.245	21.1	72.5
6	8	Menhaden Meal	12.1	1.94	1.108	22.0	67.3
7	9	Menhaden Meal	14.4	2.30	0.984	20.9	60.5
9	10	Menhaden Meal	17.8	2.855	1.297	24.7	68.6
10	12	Whole wheat 67.5 Casein 10.0 Whole milk powder 10.0	17.4	2.800	1.671	34.3	71.9
11	13	Whole wheat 35.5 Casein 5.0 Whole milk powder 10.0	10.4	1.861	1.861	37.6	85.8

The method of McCollum and Shukers gives much lower values than does that of Mitchell. The former has not yet been employed extensively. It is difficult to conceive how the results obtained by it can be far from the true values.

Free Choice as Factor of Safety in Animal Nutrition.— Osborne and Mendel (1918) studied the ability of the rat to choose between adequate and inadequate diets. They offered two types of diets to rats and determined the food ingested. The

* From studies made by C. F. Shukers for the U. S. Bureau of Fisheries, 1927-28.

diets differed in the nature of the proteins, and in one case in the vitamin B content. One diet contained lactalbumin 18 per cent., and the other zein 18 per cent. The two rats employed ate considerably more of the lactalbumin food than of the zein food, but their choice resulted in nearly normal growth. In a second experiment one diet contained 18 per cent. of casein and the other but 4.5 per cent. The selection of food differed markedly in the case of the two animals. One ate about four-fifths of the high protein diet, the other ate more than 60 per cent. of the food containing insufficient protein, and grew but little, whereas the rat which ate principally the protein-rich foods grew nearly normally. In a third experiment both diets contained 18 per cent. of protein (casein); the difference lay in their vitamin B content, one containing natural, the other artificial "protein-free milk" made of milk sugar and salts. One rat ate about two-thirds of the former, and one-third of the latter mixture, and grew well. The other selected only the vitamin-poor diet during the first 20 days, and thereafter essentially only the vitamin-rich food. It failed to grow in the first period but responded when it changed to the complete food.

Varying Food Consumption with Growth.—When a young animal is growing there is a constantly increasing need for food, and a progressive increase in consumption is observed. If two rats are fed diets alike except for their protein content, or the kind of protein, one may grow because the protein of its food is well constituted, while the other may fail because its protein is deficient. Comparison of food intake and consequently of protein consumption is not practicable in such animals, since the difference in size is becoming constantly more pronounced. Osborne and Mendel (1914-15) found that rats growing well on a diet containing 9 per cent. of casein plus a small amount of cystine, frequently ate no more calories, and ingested no more protein than did rats which were growing no better on larger percentages of casein without the addition of cystine. They concluded: "It thus appears that a marked deficiency in any essential ingredient of the diet does not lead to a corresponding compensatory increase in food intake."

Their study of the rôle of appetite in regulating food consumption led them to state: "The experience which we have gained in measuring the food eaten by many rats at all periods of their growth has given us the conviction that the intake of the individual is determined in large measure by the energy require-

ment at any given period" (1914, p. 358). To quote from page 359 of the same paper, "At any rate the animals do not consume *proportionately* more of the artificial ration because it happens to be decidedly poor in protein; but roughly speaking, they appear to limit their feeding to the amount of food yielding approximately the requisite energy." This view is in accord with that of Rubner (1902), who found that food which a dog would eagerly devour at the temperature of 0° C., would be in part refused when the temperature is raised to 33°, when his energy requirements are much lower.

Osborne and Mendel (1918) reported the food consumption of rats fed diets containing varied amounts of fat. Since fat has about double the energy value of protein or carbohydrate, much smaller amounts of the fat-rich diets would furnish the required amount of energy than would be necessary with the fat-poor diets. In all cases food consumption was found to be regulated by energy needs.

McCollum and his co-workers have throughout their studies employed diets low in fats, and very much alike in their energy values. Food intake should have been, therefore, according to Osborne and Mendel's experience, entirely comparable. Numerous records obtained in recent years have shown that this is the case. Quoting again from the paper of Osborne and Mendel (1918, p. 523)—"McCollum does not state the amount of food which his experimental rats ate; but if we assume that they ate quantities of the type of food which he employed in amounts approximately equal to those eaten by rats in our experiments on foods of corresponding calorific value, the difference between the results of his experiments and ours disappear."

Owing to faults in the technic employed in earlier experiments, we find ourselves to-day with extremely little information concerning the manner and extent to which any purified proteins supplement one another. On the other hand we have a very considerable amount of accurate knowledge concerning the supplementary relations among certain of our important food-stuffs.

Studies on Supplementary Protein Values.—With a method described above, in which an effort was made to determine the extent to which any protein or mixture of proteins falls short of the best quality yet observed, McCollum, Simmonds and Parsons (1921) made a study of simple combinations of two natural foods, supplemented with respect to all factors other than protein. In all cases the protein constituted 9 per cent. of the diet, since this

plane of intake is a critical one, even when the protein is of good quality. The life histories of rats confined to such diets show clearly whether the protein content of any diet made on the plan described should be classed as poor, good or excellent. The details of the extent to which the animals are able to grow, perform the functions of reproduction, and retain the characteristics of youth, enable one to differentiate between shades of quality in this moiety of the diet, to a degree of refinement not equalled by any other method of study.

They studied with this method the relative values of the proteins of kidney, liver, and muscle of beef as the sole source of protein. Of these, kidney is superior to the other two tissues. As sources of amino-acids liver is second, and muscle third in value.

Chart IV shows the extent to which young rats are able to grow when confined to diets comparable in all respects when the protein comes from a single source. With the exception of the diet in which milk proteins were supplied as the sole source of nitrogen, the diets described in this chart derived their protein from a single seed, either a cereal or legume seed.

Kidney, liver and milk stand out as foods containing proteins of unusual value. Among the cereal grains, wheat stands first in the quality of its protein. Rye, maize, barley, flaxseed oil meal, rolled oats, and kafir corn contain proteins of about the same quality. Barley and rye appear to be slightly better than the other seeds named.

Without exception it has been found that two cereal grains fail to supplement very well their respective protein deficiencies, so animals do little or no better when fed 9 per cent. of protein derived from two cereals than they do when confined to one. There are, however, some remarkable instances of effective supplementing between certain cereals and certain legume seeds. Conspicuous among these successful combinations is wheat and pea. When fed with all necessary additions other than protein, a group of young rats grew to the full adult size and the females were very fertile. There was a slight decrease in size in succeeding generations, but the females of the third generation still produced and reared young. In this diet wheat furnished two-thirds and peas one-third of the total proteins.

Maize and pea, fed in combination parallel to the wheat and pea ration just mentioned, was almost a failure for the nutrition of the rat. Animals confined to this diet, the only fault of which

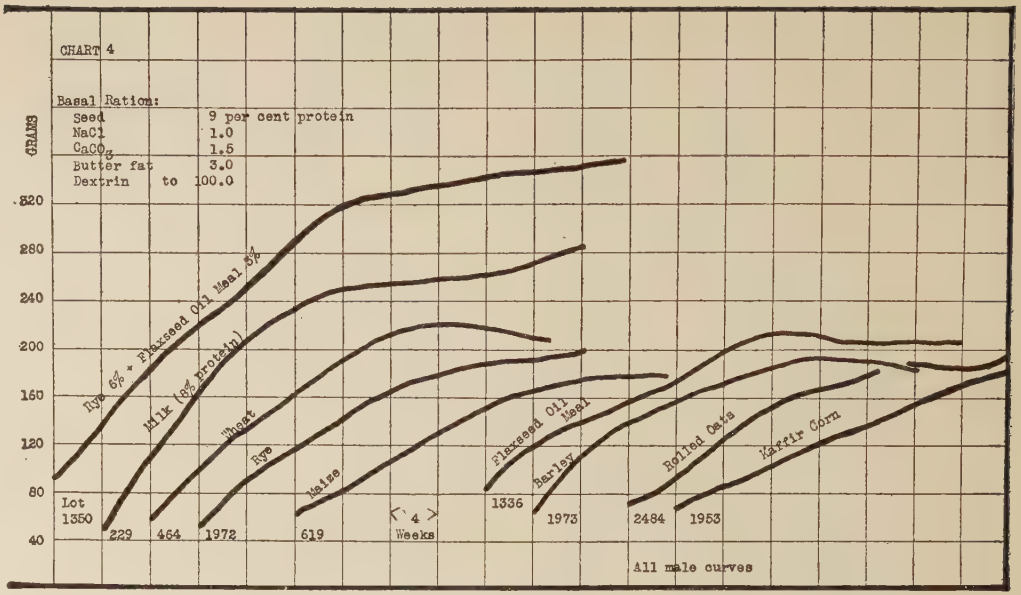


CHART. IV.—In this chart are shown a series of growth curves of rats which were fed on diets which were comparable in every respect except in the quality of their proteins. All the diets contained 9 per cent of protein except Lot 229, which contained but 8 per cent. It will be seen that rye and flaxseed oil meal furnish a combination of proteins of extraordinary nutritive value, for growth was rapid and the ultimate size attained was great. When the remaining curves are compared it will be seen that 9 per cent of wheat protein produced growth a little less satisfactory than did 8 per cent of milk proteins. None of the other proteins were as good as those mentioned. The steepness of the curve denotes the excellence of the proteins.

lay in its protein moiety, grew slowly and remained stunted. Fertility was very low, and the second generation increased very slowly in weight. See Charts V and VI.

It would take us too far afield to comment in detail upon the relative merits of all the numerous protein mixtures which were included in this study. Some of these are tabulated in condensed form in Tables X, XII, and XIV. The most striking fact brought out is that in certain cases animal tissues are more effective for enhancing the value of cereal proteins than are the proteins of milk. A mixture of rolled oats supplying 6 per cent. and milk supplying 3 per cent. of protein was inferior to a similar combination of oats and liver, but about equal in value to mixtures of oat proteins and beef muscle or beef kidney, where the cereal furnished two-thirds of the total protein and the animal tissue the remainder. Barley and milk protein likewise proved inferior to barley and animal tissue proteins. Wheat and milk, while very good as a source of proteins when combined in the proportions adhered to in these experiments, was inferior to wheat and animal tissues. Soy bean or pea proteins appeared to be about as well enhanced by milk proteins as by the three animal tissues studied.

Supplementary Relationships among Feeding-stuffs.—Hart and Steenbock (1920), in a series of experiments with cows, kept the cereal protein constant and varied the milk protein content of the diet. A highly efficient protein mixture is not attained until the proportion of liquid milk to corn meal reaches 1:1. In this proportion the milk nitrogen constituted approximately 30 per cent. of the total in the ration. They showed (1919) that meat crisps or fish meal, fed with starch and serving as the sole source of nitrogen, had an efficiency of but 40 per cent., whereas milk protein under similar conditions had a value of 60. The protein mixture from corn and milk, or oat and milk, had values of 60-65 per cent., with approximately 25 per cent. of the nitrogen derived from skim milk, whereas the efficiency of the corn and tankage mixture, or a barley and tankage mixture, was about 40 per cent. Their results show that "either milk or meat will supplement the proteins of the various cereal grains to approximately the same degree."

Hart and co-workers (1916, 1920, 1921) studied the supplementary relations of the proteins of various concentrates with those of alfalfa and corn silage, and also those of barley with alfalfa and corn silage. They used dairy cows, and the efficiency

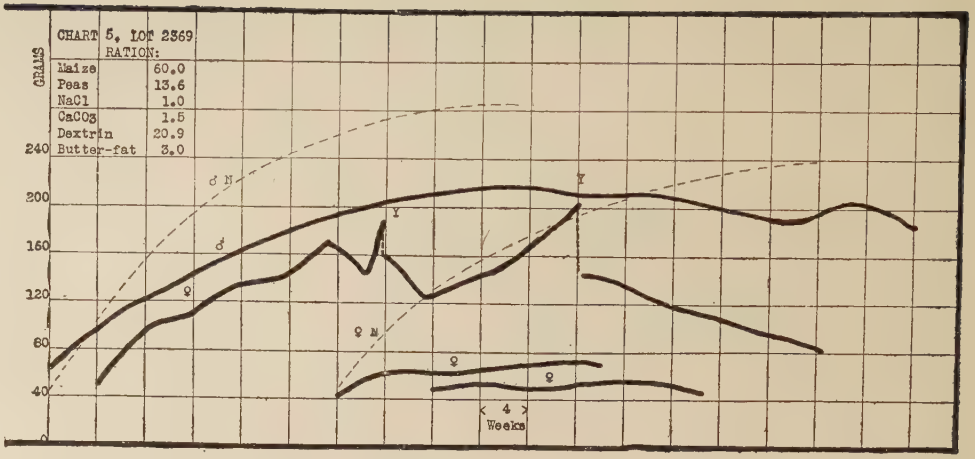


CHART V.—This chart shows the amount of growth which it is possible to induce in a rat which is confined during growth to a diet containing but 9 per cent of protein derived from maize and peas. All the deficiencies of a mixture of maize and pea were made good by the addition of the inorganic salts and butter fat. The growth was stunted, and fertility low. The young born of the rats which had grown on this diet were never able to grow after they were weaned, and lived only five to six months. This chart should be contrasted with Chart VI in which the diet was essentially similar, except that the proteins were derived from a mixture of wheat and peas instead of maize and peas. The former mixture gives proteins of much greater nutritive value than does the latter combination. It is a matter of great practical importance to so combine our natural foods as to secure protein mixtures of high biological value. (See Table XIV, p. 94.)

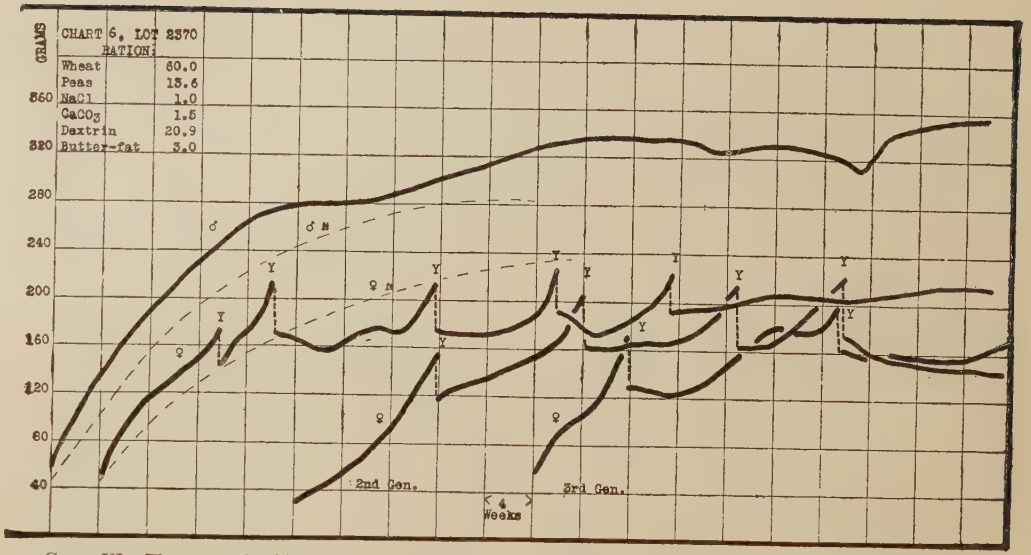


CHART VI.—The curves in this chart should be compared with those in Chart V. The two diets were alike in their biological value except in the quality of their proteins. Lot 2370 derived its proteins from wheat and peas and were able to grow well and showed high fertility because this combination contains proteins which supplement and enhance each other. Maize and pea proteins do not, on the other hand, enhance each other's values when they are combined. In animal production as well as in human nutrition, it is very important that the proteins of the diet have a high biological value.

TABLE X

SHOWING THE EFFECT ON THE GROWTH OF THE NURSING YOUNG OF DIFFERENT QUALITY OF PROTEIN IN THE MOTHER'S DIET

No. of Experiment	Wt. of Mother After Birth of Young. Grams.	Wt. of Mother 10 to 22 Days Later.		Wt. of Mother at End of Nursing.		No. of Young.	Weight of Young at Different Ages.					
		Days.	Grams.	Days.	Grams.		Days.	Grams.	Days.	Grams.	Days.	Grams.
2369 *	144	18	150	46	120	4	18	71	31	107	46	123
2069 †	215	10	232	22	205	4	10	100	22	197	..	...
2370 ‡	192	12	170	37	170	5	12	74	33	160	37	175
2153 §	175	15	182	28	192	5	15	137	28	277	..	...
2365 ‖	173	18	157	45	146	5	18	70	30	102	45	210
2186 ¶	212	22	195	33	195	6	14	125	22	184	33	280
2767 **	270	19	275	29	270	6	19	163	29	330	..	...
2370 ‡	177	20	145	44	175	7	12	81	33	167	44	250
1236 ‡‡	142	..	...	40	146	7	16	133	30	286	40	544
2186 §§	215	17	213	45	215	9	11	123	17	153	45	382
2811 ‖‖	205	19	225	28	215	10	19	225	28	387	..	...

* Diet contained 9 per cent of protein from maize and peas. (Formula on p. 92.)

† Diet consisted of milled cereal products, legume seeds, beefsteak, a salt mixture and cabbage. (Formula p. 92.)

‡ Diet contained 9 per cent of protein from wheat and peas. (Formula p. 92.)

§ Diet derived its protein from milled cereals, beefsteak and legume seeds. (Formula p. 92.)

‖ Diet derived its 9 per cent of protein from barley and navy beans. (Formula p. 92.)

¶ Diet contained 9 per cent of protein derived from wheat and beefsteak. (Formula p. 92.)

** Diet derived its protein from whole cereal grains, legume seeds and casein. (Formula p. 92.)

‡‡ A high protein diet in which the protein was furnished by 50 per cent of beefsteak. This was supplemented so as to make good all its dietary deficiencies. Note the rapid and vigorous growth of the young. The formula for this diet is given on p. 92.

§§ Diet contained 9 per cent of protein derived from wheat and beefsteak. Although the protein content was low, the quality was so high that the young made remarkable growth. (Formula of diet, p. 92.)

‖‖ Diet contained a liberal amount of protein derived from cereal grains, legume seeds, casein and milk. Note the remarkable growth of the young during the nursing period. (Formula of diet, p. 92.)

TABLE XI

COMPOSITION OF DIETS DESCRIBED IN TABLE X

No. of Exp.	*Wheat, Grams.*	*Maize, Grams.*	*Barley, Grams.*	*Rolled Oat, Grams.*	*Bolted Wheat Flour, Grams.*	*Corn Meal,* Grams.*	*Rice, Grams.*	*Pea, Grams.*	*Navy Bean, Grams.*	*Beefsteak, Grams.*	*Cabbage,† Grams.*	*Dextrin, Grams*	NaCl, *Grams.*	$CaCO_3$, *Grams.*	*Butter Fat, Grams.*	*Casein, Grams.*	*Milk Powder,‖ Grams.*	*Cod Liver Oil, Grams.*
2369		60.0						13.6				20.9	1.0	1.5	3.0			
2069				8.0	30.0	15.5	8.0	8.0	8.0	10.0	10.0		1.0	1.5				
2370	60.0							13.6				20.9	1.0	1.5	3.0			
2153				9.5	30.0	19.5	9.5	9.5	9.5	10.0			1.0	1.5				
2365			50.0						13.6			30.9	1.0	1.5	3.0			
2186	60.0									4.1		30.4	1.0	1.5	3.0			
2767	30.0	19.5		9.5			9.5	9.5	9.5				1.0	0.5		10.0		1.0
2370	60.0							13.6				20.9	1.0	1.5	3.0			
1236 ‡										50.0		43.5	1.0	1.5	3.0			
2186	60.0									4.1		30.4	1.0	1.5	3.0			
2811	25.0	11.5		9.5			9.5	9.5	9.5				1.0	1.5	3.0		10.0	

* Degerminated corn meal.
† Cabbage desiccated, steamed and dried.
‡ Contained 1.0 per cent of KCl.
‖ Merrill-Soule Company's "Klim."

TABLE XII

Supplementary Relations Between Proteins from Different Sources

ALL DIETS CONTAINED 9 PER CENT OF PROTEIN, TWO-THIRDS OF WHICH WAS DERIVED FROM THE VEGETABLE, AND ONE-THIRD FROM THE ANIMAL TISSUE OR FROM MILK

No. of Experiment	*Sources of Protein*	*Observations on Growth, Fertility, Infant Mortality*
2193	Wheat 60, kidney 4.2 per cent.	Growth excellent; reproduction good; infant mortality low.
2191	Navy beans 27.2, kidney 4.2 per cent.	Growth fair; far superior to growth on 9 per cent of bean protein.
2179	Wheat 60, liver 4.1 per cent.	Growth good; high fertility but very high infant mortality.
2177	Navy beans 27.2, liver 4.1 per cent.	Same as Lot 2191.
2182	Rye 50, liver 4.1 per cent.	Growth excellent; fertility good; infant mortality high.
2176	Peas 27.2, liver 4.1 per cent.	Growth fair; far superior to that on 9 per cent of pea protein.
2181	Oats 40, liver 4.1 per cent.	Growth good; infant mortality high.
2188	Oats 40, beef muscle 4.1 per cent.	Growth not so satisfactory as Lot 2181; fertility good; infant mortality 100 per cent.
2189	Rye 50, beef muscle 4.1 per cent.	Growth good; about like Lot 2182; fertility good; infant mortality high.
2186	Wheat 60, beef muscle 4.1 per cent.	Growth excellent; reproduction good; infant mortality high.
2387	Oats 40, milk powder 9.3 per cent.	Growth good; inferior to Lot 2181; similar to Lot 2188; fertility good; infant mortality high.
2391	Barley 50, milk powder 9.3 per cent.	Growth fair; inferior to barley and liver, kidney or muscle.
2389	Soy bean 16.6, milk powder 9.3 per cent.	Similar to results with rations of soy beans with animal tissues.
2390	Peas 27.2, milk powder 9.3 per cent.	Similar to Lot 2176; results same as with peas with kidney or beef muscle.
2384	Wheat 60, milk powder 9.3 per cent.	Results not so good as with wheat with animal tissues.

The proteins of seeds are supplemented and enhanced better by animal tissues (meats) than by milk proteins. In all cases milk proteins enhance the proteins of seeds, however, and milk has the advantage of greater superiority as a supplement in respect to inorganic elements and is far superior to muscle meats as a source of vitamin A. (For further details see Charts showing growth curves.)

TABLE XIII

COMPOSITION OF RATIONS DESCRIBED IN TABLE XII

No. of Exp.	*Wheat Grams.*	*Oats Grams.*	*Rye Grams.*	*Barley Grams.*	*Navy Bean Grams.*	*Soy Bean Grams.*	*Pea Grams.*	*Kidney Grams.*	*Liver Grams.*	*Beef Muscle Grams.*	*Milk* Powder Grams.*	*Dextrin Grams.*	NaCl *Grams.*	$CaCO_3$ *Grams.*	*Butter Fat Grams.*
2193	60.0							4.2				30.3	1.0	1.5	3.0
2191					27.2			4.2				63.1	1.0	1.5	3.0
2179	60.0								4.1			30.4	1.0	1.5	3.0
2177					27.2				4.1			63.2	1.0	1.5	3.0
2182			50.0						4.1			40.4	1.0	1.5	3.0
2176							27.2		4.1			63.2	1.0	1.5	3.0
2181		40.0							4.1			50.4	1.0	1.5	3.0
2188		40.0								4.1		50.4	1.0	1.5	3.0
2189			50.0							4.1		40.4	1.0	1.5	3.0
2186	60.0									4.1		30.4	1.0	1.5	3.0
2387		40.0									9.3	45.2	1.0	1.5	3.0
2391				50.0							9.3	35.2	1.0	1.5	3.0
2389						16.6					9.3	68.6	1.0	1.5	3.0
2390							27.2				9.3	58.0	1.0	1.5	3.0
2384	60.0										9.3	25.2	1.0	1.5	3.0

* Half skimmed milk powder. Merrill-Soule Company's "Klim."

TABLE XIV

SUPPLEMENT RELATIONS BETWEEN PROTEINS FROM DIFFERENT SOURCES. (VEGETABLE PROTEINS ONLY.)

ALL DIETS CONTAINED 9 PER CENT OF PROTEIN. TWO-THIRDS OF THE PROTEIN WAS DERIVED FROM THE FIRST NAMED, AND ONE-THIRD FROM THE LAST NAMED FOOD-STUFF, EXCEPT LOTS 2346, 2344 AND 2345 IN WHICH EACH GRAIN FURNISHED HALF THE TOTAL PROTEIN

No. of Experiment	*Source of Protein*	*Observations on Growth, Fertility and Infant Mortality*
2367	Peas 27.2, Navy beans 13.6 per cent.	Very slow growth; permanent stunting. True of all combinations with legume seeds.
2383	Navy beans 27.2, soy beans 8.3 per cent.	Same as Lot 2367.
2346	Wheat 45, rolled oats 30 per cent.	Good growth; fertility low; long nursing periods; young inferior.
2344	Wheat 45, maize 45 per cent.	Growth fair; not so good as Lot 2346. Fertility low and nursing period long. Young inferior.
2345	Maize 45, oats 30 per cent.	Growth about like Lot 2346. Fertility high and infant mortality high.
2381	Barley 50, soy beans 8.3 per cent.	Good growth; fertility low; young inferior.
2378	Wheat 60, soy beans 8.3 per cent.	Same as Lot 2381.
2365	Barley 60, navy beans 13.6 per cent.	Good growth; fertility good; long nursing periods; infant mortality high.
2380	Oats 40, soy beans 8.3 per cent.	Fair growth; not so good as Lot 2381; fertility low.
2369	Maize 60, peas 13.6 per cent.	Fair growth; not nearly so good as Lot 2370. Fertility low. Nursing periods long. Young inferior.
2372	Oats 40, peas 13.6 per cent.	Good growth; much better than Lot 2369. Not so good as Lot 2370. Nursing periods long. Mortality high.
2370	Wheat 60, peas 13.6 per cent.	Very good growth; fertility good; infant mortality quite high. Nursing periods long. This combination is the best of all cereal and legume mixtures studied.

Cereal grains enhance each other's proteins better in all cases than do combinations of proteins of two legume seeds. Neither of the above are equal in the quality of their proteins to certain combinations of a legume seed with a cereal in which the cereal furnishes two-thirds and the legume seed one-third of the total protein diet.

In Tables XII and XIV the diets were satisfactorily constituted with respect to all factors other than proteins, so the results of the experiments described turned entirely on the biological value of the proteins of the rations. The formulas for the rations used in these experiments are given in full on pages 94 and 96.

TABLE XV

COMPOSITION OF DIETS DESCRIBED IN TABLE XIV

No. of Experiment.	*Wheat grams.*	*Maize grams.*	*Oats grams.*	*Barley grams.*	*Peas grams.*	*Navy Beans grams.*	*Soy Beans grams.*	NaCl *grams.*	$CaCO_3$ *grams.*	*Dextrin grams.*	*Butter Fat grams.*
2367					27.2	13.6		1.0	1.5	53.7	3.0
2383						27.2	8.3	1.0	1.5	59.0	3.0
2346	45.0		30.0					1.0	1.5	20.5	3.0
2344	45.0	45.0						1.0	1.5	5.5	2.0
2345		45.0	30.0					1.0	1.5	20.5	2.0
2381				50.0			8.3	1.0	1.5	36.2	3.0
2378	60.0						8.3	1.0	1.5	26.2	3.0
2365				50.0		13.6		1.0	1.5	30.9	3.0
2380			40.0				8.3	1.0	1.5	46.2	3.0
2369		60.0			13.6			1.0	1.5	20.9	3.0
2372			40.0		13.6			1.0	1.5	40.9	3.0
2370	60.0				13.6			1.0	1.5	20.9	3.0

of these proteins for milk production was observed. A ration composed of 7 parts of rolled oats and 7 parts of oat straw was inadequate for cows.

Osborne and co-workers (1920) have studied the proteins of the spinach leaf. They found that a mixture of skim milk and yellow corn supplemented by some green food was adequate for young animals, although it was necessary to add calcium carbonate and sodium chloride to induce rapid growth.

Macy and Mendel (1920) fed rabbits, guinea pigs and pigeons on cottonseed kernel and found it to produce injury, the most characteristic clinical symptoms of which were emaciation, loss of appetite, weakness, rough hair and unkempt appearance, disturbance in breathing, and finally coma and perhaps paralysis.

Osborne and Mendel (1913 and 1915) found egg white gave adequate growth in rats when they were given 18 per cent. of the protein. Recently egg albumin as a source of protein has been investigated in several laboratories. Conflicting results have been reported, making further study necessary before conclusions can be drawn. There are also conflicting reports concerning the digestibility of raw egg white. Rose and MacLeod (1920) found the coefficient of digestibility for cooked egg white to be 82 to 86 per cent., for unbeaten raw whites 71 per cent., for beaten raw whites 83 per cent., and for half beaten raw whites, 81 per cent.

Cajori (1921) found that rats grew at a moderate rate on a diet containing proteins from the pecan. He believes the tannin in this nut to be a limiting factor.

"Pink Disease" in Rats and Children.—Swift (1915) described a nutritional disease now known as Swift's disease, erythroedema polyneuritis, or colloquially as "Pink Disease." This syndrome was recognized in America by Weston, and in England by Thursfield and Paterson. Cases have been reported from France, Holland, Switzerland, and Sweden.

The disease occurs in infants and young children aged 4 months to 3½ years, and most commonly between 9 months and 2 years. The majority of the children are breast-fed but some have been on a mixed diet. The condition is not infectious. It is generally afebrile and many cases recover after some months.

Warthin has reported recovery through forced feeding. There are nutritional, cutaneous and nervous symptoms, and anorexia is constant and extreme. The disease begins with a diffuse erythema over the trunk and extremities. The hands, feet,

cheeks, nose and forehead are affected. The skin is red and swollen but there is no pitting on pressure. The redness is followed by a fine desquamation, especially on the palms and soles. The extremities are very irritable, the child scratching and tearing at them. The face is rubbed into the pillow in a burrowing manner. Ulceration of the tongue or buccal mucosa is common but not extensive. The hair usually falls out in patches or is pulled out by the child, leaving large bald areas. In rare cases, the nails become loose or drop off. The mouth is allowed to hang open and the appearance has been described as that of a young gosling. There is no actual motor paralysis, but sensation over the extremities appears to be diminished. The reflexes are either diminished or absent. The child sits in a crouching attitude, the back curved and the knees drawn up. There is a mouse-like odor. There is leucocytosis of from 15,000 to 40,000.

Boas (1927) has produced experimentally a disease in rats which presents most of the symptoms of "Pink Disease" in infants. She employed a diet complete except that the sole source of protein is supplied by fresh crude egg white which had been dried. A diet consisting of dried egg white 100 gm., wheat starch 250 gm., salt mixture 185 (McCollum, Simmonds and Pitz), 25 gm., cotton seed oil 75 gm., lemon juice 25 c.c., Marmite* 25 gm., and distilled water 300 c.c., supplemented with 5 drops daily per rat of cod liver oil, produced the significant lesions during the fifth week, and nervous symptoms at the end of the sixth week. She describes the symptoms as follows:

For the first two or three weeks young rats grow well, then red scaly patches appear at the corners of the mouth, the coat becomes rough and sticky and the long hairs fall out. The fur on the abdomen shows at first a characteristic ribbed appearance, followed by the development of bald areas. The red patches spread to other parts, appearing as eczematous dermatitis. Skin hemorrhages are sometimes seen. The region around the mouth is always the most severely affected, although there is often such severe blepharitis that the eyes are closed. Hair loss is extensive and edema of the feet sometimes occurs. The rats have a distinctly musty smell. Body weight remains stationary for a week or two, but falls slowly during the second stage of the disease. Nervous symptoms appear after 6 weeks. There is pronounced spasticity of the limbs, particularly of the hind legs, and the back is arched. The rat assumes a kangaroo-

*This is autolyzed yeast; it is known in this country as Vegex.

like posture, or a crouching attitude and displays swimming movements with the front paws. Death is preceded by rapid loss of weight, and the animals show signs of extreme cyanosis. Rigor mortis sets in rapidly. At post mortem there is an almost complete absence of fat and the skin is infiltrated and vascularized. The organs seem normal.

Findlay, Marshall and Stern (1929) have repeated and confirmed the results of Boas, and state that the disease may be produced by feeding the mother rats on dried egg white as the sole source of protein. This suggests that in the drying process some essential dietetic factor in egg white is destroyed rather than the formation of a toxic substance. Their results suggest a hitherto unknown dietetic factor, since there is a close analogy to the occurrence of the diseases, beri-beri and scurvy in breast-fed infants. In rats suffering from "Pink Disease" after egg white feeding, there are characteristic changes in the central nervous system which are strikingly similar to those of Swift's disease in children. The similarities of the disease in infants and rats are summarized as follows: Both diseases occur in young animals. Swift's disease has never been recorded in adults, while in the adult rat it is only after from three to five months that cutaneous lesions can be produced. The disease may occur on a diet of mother's milk or on a ration containing ample supplies of the known vitamins. The clinical symptoms of both diseases are nutritional, nervous and cutaneous. In rats, there is a characteristic "kangaroo" position; in children, a knee-elbow position. In rats and children there is a curious mousy odor. Death is often due to an intercurrent broncho-pneumonia. There is no evidence that the diseases are due to an infection with bacteria or ultramicroscopic viruses. The pathological changes in the skin and nervous system are similar.

Boas says that raw potatoes, yeast, raw white of egg, and egg yolk were all capable of preventing the onset of this condition, whereas Marmite was not effective. Cow's milk is not very potent; 5 c.c. of summer milk daily per rat was necessary for a cure. Raw liver feeding was most effective.

Proteins of Legume Seeds.—Sure (1920 and 1921) studied the nature of the deficiencies of the proteins of certain legume seeds. He found that arachin, a protein from the peanut, did not supplement the proteins of the Georgia velvet bean. He found the same to be true of proline alone, or proline, cystine and tryptophane; likewise gliadin, zein and gelatin did not improve

these proteins. On the addition of cystine to each combination, enhancement was marked only in the bean-gelatin combination (1923).

Embrey (1921) found mung beans to supply enough protein for normal growth in mice. The protein of white kao-liang is better than that of red kao-liang, but neither seems to be adequate for growth.

Johns and co-workers (1920) state that proteins of the beans phaseolus vulgaris, phaseolus lunatus and phaseolus angularis are deficient in cystine. They found (1921) the proteins of the yellow pea (Pisum sativum) did not require extra cystine. Cystine is the limiting factor for the proteins of lima bean (1922) and adsuki bean (1921). The limiting deficiency of the lentil (Lens esculenta) is cystine (1924). Protein mixtures from the Chinese or Georgia velvet bean were inadequate for nutrition, and were not completed by adding cystine (1922). These beans contain a toxic substance. Proteins from the palm kernel were adequate for normal growth when the meal constituted 80 per cent. of the diet, and furnished 15.5 per cent. of protein (1923). They found bread made from 25 per cent. of soy bean flour and 75 per cent. of wheat flour, or from 15 per cent. of soy bean flour and 85 per cent. of wheat flour, supplied proteins and vitamin B in amounts adequate for the normal growth of rats (1921). The mixed proteins were 2 to 3 times as efficient for growth as wheat proteins alone.

A diet containing only the proteins of tomato press seed cake supported normal growth in mice when the protein constituted 17.2 per cent. of the food (1921).

Sure (1921) sought to find which amino-acid in zein causes it to enhance the proteins of the pea. He confirmed the findings of McCollum, Simmonds and Pitz that zein supplements pea proteins (Vicia sativa), whereas lactalbumin and gelatin do not. Neither arachin nor edestin (hemp-seed) improves pea proteins. No improvement was seen in pea proteins when these were supplemented with leucine, or with the "leucine fraction" which contains leucine, valine and alanine, all of which are digestion products of proteins (1921). Tryosine alone or with leucine and cystine did not enhance pea proteins, so he drew the conclusion that "the benzene ring must be eliminated as the nucleus responsible for the deficiency of the proteins in question." He also states (1921), "Proline, even in the absence of alanine, leucine, valine, cystine and tyrosine, did not supply the missing

link furnished by zein in improving the poor quality of the proteins of the pea." These experiments eliminate alanine, leucine, valine, tryptophane, lysine, cystine, tyrosine and proline as being the amino-acids which are responsible for the deficiency of the proteins of the pea. Sure says, "Since zein, which supplements the pea, is very low in arginine and histidine, it is thought possible that zein may contain one or more amino-acids which we do not know about." Sure (1922) found that neither lactalbumin, arachin nor zein improve edestin. Edestin fed at 12-18 per cent. of the ration, even when carrying 2 per cent. of the total nitrogen in the form of an alcoholic extract of wheat germ, did not suffice for growth. Edestin was enhanced by cystine only when accompanied by lysine. Sure (1922) using edestin, added cystine, lysine and arginine without marked enhancement. In another experiment with this mixture he added proline and in another pyrrolidone carboxylic acid. He concluded that proline is essential for growth, and that the animal has not the capacity to transform pyrrolidone into pyrrolidine carboxylic acid, or into proline.

Salmon (1922) fed mature pigeons dried ground velvet beans and found that on the second day all showed rough feathers and a sleepy appearance. Several died after 4 or 5 days. Plimmer and Rosedale (1922) found casein and lactalbumin to form a good source of proteins for growing chicks.

McCandlish and Weaver (1922) studied the comparative values of old process linseed oil meal, peanut meal, soy bean meal and wheat gluten as protein supplements in the rations of dairy cows. Coconut meal had a higher value than the others despite its low protein content. In palatability they found the feeds to range: linseed oil meal, soy bean meal, gluten feed, peanut meal, and coconut meal.

Maynard and co-workers (1923) found combinations of peanut oil meal and rice bran superior to corn meal proteins alone, but a mixture of corn meal and rice bran was even better. The supplementary relation of peanut meal and corn meal is not marked. Maynard employed the method of feeding diets containing the critical content of 9 per cent. of protein.

Shiba and Koyama (1923) found that rats grew less with soy bean protein than with peanut protein. Eddy and Eckman (1923) found that peanut flour proved slightly superior to meat for growth and markedly superior for reproduction, as a supplement to the proteins of wheat flour. Morgan and co-workers

(1923) state that the proteins obtained from ground blanched almonds after removing the oil supported normal growth in mice when the protein constituted 17.2 per cent. of the food.

Investigations Relating to Sulphur Metabolism.—Several investigations have recently been reported of attempts to replace cystine by other sulphur compounds. Lewis and Lewis (1927) demonstrated that flowers of sulphur cannot replace cystine. Flowers of sulphur are definitely toxic, causing the death of rats when 1 per cent. is included in the diet, presumably because of the formation of hydrogen sulphide in the intestine. Westerman and Rose (1927) found synthetic dithiodiglocollic acid and β-dithiodipropionic acid cannot replace cystine in the diet.

Studies on the Synthesis of Amino-Acids in the Body.—McGinty, Lewis and Marvel (1924) found α-hydroxy-ε-caproic acid, which is lysine with the amino group replaced by a hydroxyl group, could not replace lysine in the diet. They demonstrated that the conversion of α-hydroxy acids into the corresponding amino-acids is not a general reaction, although possible in the synthesis of histidine.

An excellent study by Cox and Rose (1926) demonstrates that imidazole, 4-methyl imidazole, 4-hydroxyimidazole, 4-imidazole formaldehyde, 4-imidazole carboxylic acid, 4-imidazole acetic acid, 4-imidazole propionic acid and β-4-imidazole acrylic acid cannot replace histidine in the diet. The addition of d, 1-β-4-imidazole lactic acid to a diet complete except for lack of histidine caused an immediate resumption of growth in rats. This experiment demonstrates the synthesis of the amino-histidine from the corresponding hydroxy compound. Rose and Cox (1926) also demonstrated that arginine cannot replace histidine in the diet. The latter is an indispensable complex. Adenine, guanine, creatinine, creatine, or a combination of these could not replace histidine in the diet.

Harrow and Sherwin (1926) discuss the synthesis of histidine in the body. It may be replaced to a certain extent by imidazole pyruvic acid, but imidazole lactic acid is more efficient. Imidazole acrylic acid is used to a slight extent for conversion into histidine. Their experiments support the view that the first step in the metabolism of histidine is the formation of the α-keto or the α-hydroxy acid and that the reaction is reversible. Imidazole alone is without value for the synthesis of histidine. Sherwin is the outstanding student of the kinds of transforma-

tions which the body can accomplish with many different kinds of substances with known structure.

Jackson (1927) has conducted analogous experiments with 1-β-3-indole lactic acid in an attempt to show whether the hydroxyl group can be replaced by an amino group, converting it into tryptophane, but without success. Bunney and Ross (1928) have reëxamined this problem in the light of accumulated experience in the conduct of experimental feeding, and have decided that since rats can grow well on a diet practically devoid of arginine, this amino-acid is not essential in the diet. They secured rapid growth of rats on a diet very poor in glutamic and aspartic acids.

A Biological Assay of Proteins for Cystine.—Sherman and Woods (1925) and Sherman and Merrill (1925) have described a procedure whereby graded additions of cystine are added in a series of rat experiments to a basal diet in which cystine is the limiting factor for growth. They estimate that casein contains not less than 1.3 per cent. and not more than 2.5 per cent. of cystine. Their results are higher than are those of chemical analysis. Not only in casein, but in the protein mixture of whole milk, cystine is the amino-acid present in amount so small as to be the limiting factor for growth. Sullivan (1925) has made the interesting observation that the tissues of pigeons in severe polyneuritis contain little if any cysteine, but do contain cystine; while on a rice diet plus vitamin B the tissues contain cysteine as well as cystine.

Woods (1925) states that the tissues of rats stunted for lack of cystine present a strikingly good appearance at autopsy as compared with those of animals stunted by vitamin deficiency.

Lewis (1925) administered the sodium salt of cystine orally in doses of 0.5 to 1.0 gm. per kilogram for successive days to fasting rabbits and to rabbits fed oats and cabbage. Protein casts appeared in the urine, the excretion of non-protein nitrogen and creatinine were depressed, but there was no marked loss of cystine as evidenced by the cystine and amino-acid content of the urine. The blood showed a marked rise in non-protein nitrogen. These studies are of great interest because they demonstrate that excessive doses of this normal digestion product of proteins cause experimental nephritis. Sherwin and co-workers (1925) found that cystine and cysteine compounds fed to rabbits when in sulphur equilibrium increased the sulphur in the urine. The evidence indicates that these compounds disturb sulphur metab-

olism and that some of the excreted sulphur is derived from the body tissues.

Lewis and Lewis (1926) and Beard (1926) found taurine unable to replace cystine, and the first mentioned investigators showed the same for cysteinic acid.

Hartwell (1923) believes that darkening of the coat of rats is brought about by feeding large quantities of proteins, rich in tyrosine and tryptophane. She states, "These experiments support the view that the melanins are formed from tryosine and tryptophane."

These studies of the effects of lack of individual amino-acids on the body are introduced here because of their bearing on the nature of the injury due to such lack. The effects are not specific and characteristic as are the deficiency diseases resulting from lack of each of the vitamins.

Tryptophane and Thyroid Activity.—Several investigations have been directed toward determining whether there is any relation between the tryptophane ingestion and the activity of the thyroid gland. For some years it was generally accepted that thyroxin, the active principle of the thyroid, was an iodized derivative of tryptophane, a view now known to be erroneous. Chang (1925) found that an excessive ingestion of tryptophane did not cause thyroid enlargement, and concluded that the general nutrition rather than the tryptophane in the food is responsible for changes in the thyroid gland. Abel and co-workers (1925) also found the malnutrition resulting from lack of tryptophane to involve the tissues generally and to be largely extra-thyroid. Hicks (1926) also concluded that there is no relation between tryptophane supply and thyroid hormone production. He estimates that the minimum quantity of tryptophane in the diet is about 0.5 per cent. of the protein fraction.

In this chapter an attempt has been made to make clear the types of experiments which have been employed for the study of the differences in the nutritive values of proteins from different sources, and to determine the exact nature of the limiting deficiencies of each.

CHAPTER VIII

THE PHYSIOLOGICAL REQUIREMENTS OF MAN FOR PROTEIN

The observations in the preceding chapters which show the profound damage which results from lack of sufficient of the essential mineral elements or of the vitamins, illustrate how complicated is the problem of interpreting quality in a diet. One can never safely focus attention upon a single factor. A great deal of detailed knowledge of the chemistry of food-stuffs, of the nutritive needs of the body, and of experimental studies, is essential to the formation of safe judgments regarding any problem in nutrition.

With these data in mind we may turn to the studies which have been conducted upon human subjects and evaluate them anew in the light of newer observations on animals. Outstanding among nutrition studies on man are those of Chittenden (1904 and 1907) and of Benedict (1906 and 1918). Since far-reaching deductions have been drawn from these results, a few words of comment are called for in reference to their trustworthiness as a basis of guidance in deciding upon a safe dietary regimen for man.

Physiological Economy in Nutrition.—Chittenden conceived the idea that a low protein dietary, when it provides a suitable variety, will best meet the nutritive requirements of the body. He emphasized the desirability of relieving the organs of the task of degrading daily more protein than is necessary for the repair of tissue waste. He also emphasized the fact that the body does not tend to store protein which is ingested in excess of the daily need; and that an excessive protein ingestion promotes putrefactive decomposition in the intestine, which results in the absorption of products which are physiological abominations. The logical deduction was that the protein intake should be reduced to as low a level as possible, and yet keep the body in nitrogen equilibrium.

In order to demonstrate whether such a regimen was safe for

the adult, Chittenden tested upon himself the proposition which had hitherto been accepted on the authority of Voit and of Atwater, viz., that the average adult at medium work requires about 116-120 gm. of protein per day. He then reduced his protein intake to about one-third this amount and felt better and was clearer mentally than he had been on a more liberal protein dietary. Brief comment has already been given of the experimental demonstration which he planned and carried out during 1903-04 at Yale University, in which a group of faculty men, a group of students and a group of soldiers participated. During a period of nine months the men reduced their protein intake to about half the accepted requirements. They appeared to be in excellent physical condition at the end of the period of observation. The deduction was drawn that such a dietary regimen appeared to be suitable for the maintenance of well-being in the adult for an indefinite period.

Later experiments of Benedict (1918) covered the period of a school year. The subjects were volunteer college students. He was led to abandon his earlier view that a liberal protein dietary is best for the promotion of health, and accepted the dietary principles enunciated by Chittenden.

High Protein Dietary and Kidney Damage.—The view has long been popular that metabolism of large amounts of protein which necessitates the excretion of large amounts of waste products may cause damage to the kidneys. Squire and Newburgh (1921) discussed renal irritation in man on a high protein diet. Newburgh and Clarkson (1923) found that most rabbits which ate a diet containing 27 per cent. or more of protein (meat) for 6 months or longer, developed a chronic nephropathy. The lesion, they believe, may in part be caused by infection acting on a kidney made more susceptible to infection by diet. They suggested that the injury may be due to excessive excretion of certain amino-acids, or to a combination of these and the acidity of the urine; they found that acidity alone does not cause such injury. Drummond and co-workers (1922) fed a diet containing 83 per cent. of casein, supplemented with other dietary essentials. Their rats grew at a subnormal rate and did not breed, but appeared to be in good health. The weights and microscopic appearance of the internal organs were normal; body fat was present, but the ribs were beaded. Intestinal putrefaction was excessive. Kittens fed raw lean beef with yeast, a salt mixture and shark liver oil showed beading of the ribs but no other

abnormality. It seems probable that their rats and kittens were suffering from what McCollum, Simmonds, Park and Shipley have designated as high phosphorus rickets, since their diets were excessively rich in phosphorus and poor in calcium. Their animals apparently did not live long enough to develop kidney lesions. Polvogt, McCollum and Simmonds (1923) fed rats for periods varying from 250 to 400 days on diets containing 31 to 41.3 per cent. of protein. The protein was derived in great measure from combinations of liver and casein or beefsteak and casein. Their diets were satisfactory in their composition with respect to factors other than protein. All animals had lesions of the kidneys of considerable severity. Even comparatively young animals of the fifth generation of a family confined to a high protein diet showed lesions. There was intense congestion and hyaline casts in large numbers. Osborne and Mendel (1921) fed 90 per cent. of protein (casein) in a diet otherwise complete. Even with such foods almost devoid of carbohydrates animals made satisfactory growth to maturity. Osborne, Mendel, Park and Darrow (1923) fed rats high protein (casein) diets and noted hypertrophy of the kidneys but no nephritis, and no inflammatory or degenerative changes.

Newburgh and Marsh (1924) suggested that renal injury might at least in part be caused by the passage of unusually large amounts of some amino-acids through the kidney. To test this hypothesis, amino-acids were injected intravenously into rabbits and dogs. Glycocoll, alanine, leucine, glutamic acid, arginine and phenylalanine in doses up to 5 gm. per kilo caused no demonstrable injury. On the other hand, histidine, tyrosine and cystine in single injections of less than 1 gm. per kilo caused albuminuria and cylindruria even in dogs. Sections of the kidneys showed acute parenchymatous injury. The results with tryptophane, aspartic acid and lysine are still in doubt. Serine and proline have not been tested. This discovery is of extraordinary interest in connection with the ability of the kidney to excrete certain complexes derived in the normal course of digestion from protein without injury, whereas others exerted destructive action upon that organ.

Moise and Smith (1927) review the literature on this subject and report further work on the effect of high protein diets on the kidneys. The recent work of Newburgh and Curtis (1928) appears to afford a satisfactory explanation for the discrepancies in the results of other workers. Since Polvogt, McCollum and

Simmonds had reported severe kidney lesions in rats fed high protein diets (casein plus liver), and since others had not been able to confirm this work, using casein alone Newburgh and Curtis studied the effects of feeding high protein from several sources. They also gave large single injections of individual amino-acids and brought to light an important fact. They employed diets containing 12, 18, 25, 32, 39, and 75 per cent. of protein. They found that although casein is slightly injurious at high levels the injury may be easily overlooked. Beef muscle or liver fed at high levels produced pronounced kidney injury. Beef muscle produces albuminuria of about ten times the intensity as the same percentage of casein, and beef liver proteins are often more injurious than muscle. No animal on the liver diet survived a year. A diet containing 70 per cent. of beef muscle fed more than a year caused a diffused connective tissue increase around the glomeruli. Kidneys of rats receiving liver were without exception granular.

These investigators emphasize that the kind of protein and the time factor are the important ones in producing experimental lesions of the kidney due to high protein feeding.

The extensive nutrition studies on animals seem to place in a new light the older literature relating to human nutrition. There is no instance in our experience where a diet satisfactory in all other respects, but supplying just sufficient protein of good quality to support growth at approximately the maximum rate to the full adult size, has been found to promote as satisfactory nutrition over the entire span of adult life as would the same diet containing a more liberal supply of the protein factor. It has been frequently assumed by students of nutrition that after growth has been attained the nutritive needs of the body for protein can safely be met by a dietary in which the protein content is lower than is essential for optimum growth. Of the numerous experimental data from the work of McCollum and Simmonds, none support this view. They all point to the conclusion that when the life history of the individual is considered, a generous protein ingestion or one allowing a fair margin of safety over the lowest percentage which just suffices to induce maximal growth in the young serves to maintain vigor for the longest possible period.

CHAPTER IX

THE DIETARY PROPERTIES OF INDIVIDUAL FOOD-STUFFS

It will be appreciated how confusing was the interpretation of the cause of failure or of success with experimental diets until recently, since a few years ago there was no definite conception of the existence of several dietary factors which are indispensable. Fortunately, the rat does not require vitamin C in the diet, so the element of freshness in food was not essential for success in feeding it. This species was of special value in gaining a working hypothesis regarding the number of vitamins essential in nutrition. Even with the rat, however, in addition to the unknown factors, there were variables in the quality of the proteins and the amounts of certain mineral elements, any one of which might determine success or failure of development. The solution of such a problem demanded first the accumulation of a considerable amount of data based upon the outcome of experiments, each of which was not possible of interpretation except in the light of all the others.

In order to make clear how foods should be combined so as to make good each other's deficiencies, the dietary properties of each of the more important natural foods will be described. This will form the subject of the present chapter.

Wheat.—This is the most important seed grain used as food by all western peoples, and is second only to rice in respect to the quantity used by man. Rice is in greatest favor among peoples in the wettest regions of the world. The dietary properties of wheat have been described in part in Chapter III in connection with the development of the biological method for the analysis of a food-stuff. The proteins of this grain are a little more efficient for promoting growth than are the same amounts of protein from milk or eggs. No cereal grain contains proteins superior to those of wheat. Its prominent place in the diet of mankind is justified by the results of experiments on animals. The quality of wheat proteins is illustrated by the comparisons

shown in Chart IV. The results of feeding wheat as the sole source of protein have not been uniformly successful; it appears that some wheats are superior to others. It is well known that the proteins in different samples of wheat vary markedly with respect to their glutinous property, so important in bread making. The reason for this has not been satisfactorily explained. It may be due in part at least to variability in the proportions among the several individual proteins in the seed. If this is true, it would account for such inequalities in nutritional value as have been observed.

Wheat is too poor in calcium to meet the needs of the growing rat. The average content of this element per 100 gm. of this cereal is about 0.041 gm. The optimum content of calcium for the growing rat is not far from 0.64 per cent. of the food mixture. Phosphorus, sodium, chlorine and iodine are also present in the wheat kernel in amounts too low to support normal nutrition in this species.

Bell and Mendel (1922) examined different wheats for their content of vitamin B, and found that Marquis spring wheat had a higher vitamin content than Minnesota winter wheat.

Hart, Halpin and Steenbock (1920) fed half-grown chickens on wheat, wheat gluten and calcium carbonate. The birds died within 3 months. In contrast, chickens fed corn meal, gluten feed (corn) and calcium carbonate made slow growth and maintained themselves. The first diet was probably too low in vitamin A to sustain life.

Variation of Grains in Inorganic Content.—There is great variation in the inorganic content of agricultural crops, depending upon the composition of the soil, the amount of water supplied, and also upon the manner of its application, especially under irrigation. A valuable study by Forbes and co-workers (1921) shows that blue grass from different localities varies greatly in its content of mineral elements. Some blue grass from Ohio contained twice as much of certain mineral elements as did other samples.

Greaves and Carter (1923) studied wheat, oats and barley, using two types of irrigation. In different experiments water was applied (a) in large amounts a few times; (b) the same quantity was furnished in small amounts at intervals. Large applications of water wash the nitrates beyond the roots and this results in low nitrogen content of the grain. The decrease for wheat was 21 per cent., for oats 40 per cent., and for barley 19 per cent.

Where water was supplied in small, frequent applications the protein content of the grain was high.

As the irrigation water was increased, the quantities of ash, phosphorus and calcium increased progressively, as shown in Table XVI.

TABLE XVI

Grain	*Per cent. increase of*		
	Ash	*P*	*Ca*
Wheat	46	55	155
Oats	31	35	22
Barley	36	30	41

Similar increases were observed for potassium and magnesium. These results confirm the earlier work of Forbes and co-workers mentioned above. Orr, Elliott and Wood (1925) have shown similar changes in the mineral content of pasture grass as influenced by character of the soil.

Greaves (1926) has further shown that wheat, oats and barley grown on irrigated land contain less protein than grains grown on non-irrigated soils, but the ash content is the reverse. He confirms his earlier findings that potassium, phosphorus, calcium and magnesium in grains grown on irrigated lands are increased. A ration of grains grown on irrigated soil might be entirely sufficient as a source of calcium and phosphorus to promote good skeletal development, whereas the same grains grown under less favorable conditions would fail to do so.

Wheat Germ.—This constitutes about 1.5 per cent. of the entire kernel. It contains about 30 per cent. of protein. The remainder of the seed, about 83 per cent., is endosperm containing about 11 per cent. of protein. McCollum, Simmonds and Pitz (1916) found wheat germ to be exceptionally valuable in several respects. Its proteins are abundant and of good quality; its content of the essential mineral elements is much greater than in bolted flour; it is exceptionally rich in vitamins B and E and contains some vitamin A. The oil, of which the germ contains about 10 per cent., leaves a somewhat unpleasant after-taste, and if fed in large quantities is somewhat injurious to animals. Evans and Bishop (1922) have shown the oil to be the best source of vitamin E. The latter is essential for reproduction.

The color of whole wheat flour is due chiefly to the presence

of bran and in less degree to a yellow pigment in the germ. In making white flour the germ and the bran are removed.

For some reason wheat germ as a stock feed has never found favor with farmers, so the pure product is not marketed for feeding. It is mixed with bran, or a low grade flour known as middlings, and reaches the feeder in this mixed form.

Bolted Wheat Flour.—In point of quantity consumed white flour is the most important energy food in America and Europe. This is important because it is notably deficient in more dietary factors than any other food entering into the diet, except sugars, starches and fats which are sold in the pure state. It consists essentially of starch, proteins and inorganic salts. Its proteins are of relatively poor quality and its mineral components are poor in calcium, sodium, chlorine, iron, and phosphorus.

Gliadin and glutenin are the principal proteins of flour. These are unlike any proteins in the body in their yield of the amino-acids. They are not constituted so as to enhance each other's value by compensating for each other's deficiencies, and cannot be effectively transformed into body proteins unless they are combined with other foods wisely chosen. Wheat flour is very deficient in all vitamins.

The merits of flour as a food rest essentially upon the peculiar glutinous properties of its proteins, which make possible the formation of dough. This is tenacious, and permits of leavening by the generation of carbon dioxid gas within it, through the agency of yeast or other leavening agent. By this means light, spongy bread is secured. Bolted flour has excellent keeping qualities and can be distributed without commercial hazard. This is a matter of great importance in a country like the United States. It will, despite its shortcomings from a dietary standpoint, remain our most important energy-yielding food.

It is not to be understood from what has been said that wheat flour is not a wholesome food: for when properly combined with other foods to make a well-balanced ration it is an entirely satisfactory component. *The keynote to the discussion of the individual foods entering into the diet is the importance of using proper combinations of foods.* Greig and Curjel (1918) found "atta" biscuits, which contain 15 per cent. of wheat germ, to be rich in vitamin B; they recommend them for emergency rations.

Ghose (1922) examined different Indian food-stuffs. Bleached (pure white) Indian flour is deficient in vitamin B, but crude "atta," which is a mixture of various flours such as wheat, peas,

maize, etc., and unbleached Indian flour, contains a considerable amount.

The attitude which one should take toward bolted and whole wheat flours is now easy to understand. The latter is decidedly more suitably constituted to maintain well-being for a short time if it serves as the sole article of diet, as it may under famine conditions. Whole wheat flour is an incomplete food and must be supplemented in order to compensate for its deficiencies. The unfortunate individual who, through faulty habits of living finds that his efficiency is diminishing and the joy of living slipping away at an age at which he should be still in possession of the full vigor of middle life, has not infrequently turned for relief to substituting whole wheat flour for the staple white variety. Such individuals would gain much more through adhering to a well-balanced diet than through adopting this or any other dietary fad.

Osborne and Mendel (1919) studied wheat bran. Its proteins have a higher value than those of the germ, and are superior to those of bolted flour.

Maize Kernel.—This cereal has dietary properties similar to wheat. Its flavor and physical properties, however, are different. Its proteins have no glutinous properties and therefore do not form a dough. In the southern United States maize is much prized as a human food. The United States Food Administration ("Food and the War," 1918) found that it constituted 23 per cent. of the total food intake of Tennessee and Georgia mountaineers, and 32.5 per cent. of that of southern negroes. Among 72 northern families in comfortable circumstances, only 1.6 per cent. of the diet was derived from maize. The bulk of the maize crop, which in this country amounted to three billion bushels in 1917, is used as stock feed.

When fed as the sole source of proteins, maize has a slightly lower nutritional value than wheat. Its mineral deficiencies are about the same as the latter. The two grains are comparable in their content of vitamin B.

Steenbock and Boutwell (1920) observed that yellow maize is superior to both the white and red varieties as a source of vitamin A. They believed at one time that vitamin A was associated with the yellow pigment of plants; but this view has been in great measure abandoned.

Maize used as human food is now freed from its germ and its bran layer for improving its appearance and especially its keep-

ing qualities. Maize germ, like wheat germ, is rich in oil which tends to become rancid when the kernel is ground and exposed to the air. Both white and yellow grain are used for meal for human consumption. Corn oil is used as a salad oil, and as a fat for cooking.

Oats.—Rolled oats are used chiefly as a breakfast food. They have long been a principal food grain in Scotland. In most respects the dietary properties of the oat kernel are comparable to those of wheat and maize. There is much less vitamin A in rolled oats than in wheat, and very much less than in yellow maize.

Oats have long been a favorite among feeders of horses, and are believed by many to have exceptionally high nutritive value because animals fed liberally with oats frequently show much spirit when driven or ridden. Rats fed restricted diets of certain types, faulty in one or more respects, become restless, irritable and apprehensive, and the thought occurs that perhaps the common expression among horsemen that an animal "feels his oats" may have been misinterpreted. The tendency of horses fed liberally on this grain to exert themselves greatly without urging may be the result of increased irritability and apprehensiveness. Scheunert (1918) fed a horse exclusively on oats. He noted that the calcium appearing in the feces was irregular from day to day and that there was a steady decrease in the urinary calcium as the experiment progressed. After 19 days the horse showed loss of appetite, was declining in weight and had a tendency to lameness.

Rice.—This is the most important cereal in the diet of more than half of the human race. It is used especially in the wettest parts of the world; in the United States it is used in relatively small amounts. Among primitive peoples rice is eaten without polishing, i. e., as red rice, but it is usually treated so as to lose a large part of its germ. This loss results from the pounding of the kernels in rude mortars. The bran layer, which is richer in mineral salts than the endosperm of the seed, is retained in this process.

Rice which is used for export and for sale in the large cities at a distance from the place of production is polished by abrasion. The abrasive action wears away the bran and germ which form rice polishings. The germ of rice, like that of wheat and maize, consists of cellular structures, and is a more complete food than any other part of the kernel. It contains almost all

the fats in the grain, and is more efficient in nourishing insects as well as higher animals than is the polished grain. Hamada (1923) reports that rich embryo protein has a high nutritive value. Unpolished rice loses its flavor in a warm climate because the fats become rancid. Polished rice can be handled without commercial hazard.

McCarrison (1923) found vitamin A in paddy or unhulled rice. The milling of raw paddy does not remove the whole content of A, so it is not confined to the peripheral layers of the grain. It is in great measure destroyed by steam passing through paddy contained in vats, as in parboiling.

The polishing of rice is done to improve its keeping quality, and the incidental whitening of the kernels led to a demand for a white product. *This, and the artificially established liking for white flour and white corn meal, is an illustration of the failure of the instinct of man to serve as a safe guide in the selection of food.* The esthetic sense is appealed to in greatest measure in this case by the products of lowest biologic values.

Attractiveness of rice to the eye is so important commercially that artificial whitening of the polished kernels has come into vogue. The kernels are coated with talcum powder, made to adhere by a thin coating of glucose. The milky appearance of water in which rice is washed is due to the talcum remaining in suspension. Rice which has been polished but not coated is called brown rice as contrasted with the coated or white rice.

Chart III shows that polished rice must be improved with respect to at least 4 dietary factors before it can become a complete food. Its proteins are of low value; it is too poor in all essential mineral elements to meet the needs of a growing animal, and is almost devoid of all the vitamins. The data in Chart III were obtained with the rat, and therefore do not bring out the fact that rice is lacking in vitamin C. This substance is not essential in the diet of the rat.

Kennedy (1924) found wild rice to contain a higher percentage of protein than most other cereals. It also resembles other cereals in containing inorganic material unsuitable for promoting growth. Its content of vitamin A is low, but sufficient to prevent xerophthalmia. Wild rice has a greater food value than the cultivated polished rice, because its proteins are of better quality. It contains adequate amounts of vitamin B for growth.

Barley, Rye and Millet.—Steenbock (1918) and Osborne and Mendel (1919) have made biological analyses of these cereals.

They have essentially the same dietary properties as other cereals. Steenbock (1923) found the vitamin content of millet variable. It appears that among millets the vitamin A content bears no definite relation to total pigment.

Peas and Beans.—These are known as legumes. By virtue of their symbiotic relation with bacteria in their root nodules, these plants are able to utilize the nitrogen of the air for synthesizing proteins. Other plants secure their nitrogen from the soil in the form of nitrates or ammonium compounds. The legume seeds are richer in protein than are cereal grains. Wheat, maize, oat and rice kernels contain about 11, 10, 15 and 9 per cent. respectively, whereas peas and beans contain about 23 per cent. of protein. For this reason the latter were long regarded as especially valuable sources of protein. Beans have been called "poor man's meat," but modern studies have not lent support to this view. From chemical analysis their proteins appear to be much more adequately constituted than those of wheat or maize, but experiments on animals show that there is something lacking in their molecules which limits the extent to which they can be converted into tissue proteins.

The foods next in importance to the cereals in the prevention of beri-beri are pulses (peas and beans). Grijns (1901) and Hulshoff-Pol (1907) demonstrated the value of certain beans (Katjang-hidjoe) in the prevention and cure of human beri-beri and avian polyneuritis. Johns and Jones and co-workers (1915 to 1921) made extensive studies of legume seeds. Read and Sure (1921-1923) found the Georgia velvet bean to be injurious to rats when 40 per cent. was included in the diet. These beans are rich in vitamins A and B. Ghose (1922) found Indian lentils a good source of vitamin B. Findlay (1924) reported that samples of Indian lentils and peas kept 38 years still contained appreciable amounts of B. Seeds which have lost their power of germination may still contain vitamin B. Jansen (1923) found rice stored as paddy for 100 years had lost little or none of its anti-beri-beri property.

Osborne and Mendel (1912) did not secure growth in rats with pea and bean proteins. Beans contain hemicelluloses which are not digestible but are easily fermented in the intestine with the formation of gas and may cause flatulence. The supplementary relations of pea and bean proteins for other proteins are discussed elsewhere (p. 95). There is little vitamin A in them. Their content of B is similar to that of cereal grains. Steenbock

(1921) found that green peas containing yellow pigment have more vitamin A than those devoid of yellow pigment.

A matter of importance in comparing values of the proteins of legume seeds with those of meats, is the extent to which each supplies an abundance of those amino-acids which are not yielded in optimum amounts by the proteins of the cereal grains and tubers. It is not so important to secure a high intake of protein as it is to have proteins of excellent quality. Relatively small additions of the right kind of protein greatly enhance the value of the proteins of cereals. Legume proteins are far inferior to meats in this respect.

Soy Beans.—Soy beans have long been important in the diet of Chinese and Japanese peoples. Adolph (1922) states that Buddhist monks subsist largely upon soy bean curd. In recent years the soy bean has been grown somewhat extensively in the United States. Daniels and Nichols (1917) and Osborne and Mendel (1917) show that this seed belongs in the same class with peas and navy beans. Its proteins are adequate when fed at a plane corresponding to 17 per cent. or more of the diet, and can support growth. The vitamin content of soy beans is essentially like that of peas and navy beans. The legume seeds as a group are deficient in calcium, sodium, and chlorine.

Peanut.—The peanut is a legume seed. In America it is eaten roasted as a confection; it is also used in the form of peanut butter. Peanuts are grown extensively for feeding hogs, but if fed liberally they produce oily pork. To avoid this animals are frequently finished on some other feed.

Daniels and Loughlin (1918) found Spanish peanuts to have the dietary properties common to seeds. Their greatest deficiencies are in their mineral content and in vitamin A. Johns and Jones (1917) showed the proteins of peanut to be rich in lysine. Anderson and Mendel (1928) have shown that when the diet of rats contains 60 per cent. of peanut oil, soft fat is produced. This is of great interest since peanuts have been used extensively in the South for feeding pigs, with the result that much soft pork has been produced. Their paper discusses the relation of diet to body fat.

Cotton-seed Flour.—In the manufacture of cotton-seed flour, the oil, hull, and a considerable amount of resinous substances are removed. The flour has a nutty flavor and an effort has been made to promote it as a human food. It has been studied by Richardson and Green (1916), Osborne and Mendel (1917),

Wells and Ewing (1916), and Macy and Mendel (1920). Like all other seeds, the proteins are complete when fed in the mixture in which they occur in the seed. The deficiencies of the product are those common to seeds, viz., lack of sufficient calcium, sodium, chlorine, and probably phosphorus and vitamin A. Osborne and Mendel (1917) found the proteins of this flour to supplement maize gluten, obtained in the manufacture of corn starch. For this purpose they are equal to the proteins of brewer's grains, but inferior to lactalbumin.

Cotton-seed, and the press cake obtained in the manufacture of cotton-seed oil, contains a poisonous substance which Withers and Carruth (1915) called *gossypol.* The oil extracted from the seed is also toxic to animals because of the presence of *gossypol.* Carruth (1917) found that cooking the meal or heating it in the hot press method for extracting the oil renders *gossypol* no longer soluble in oil or ether; it therefore remains in the meal. Although cotton-seed flour and meal are valuable feeding-stuffs for animals, their use as human foods is not recommended, since there is no assurance that their toxicity can be removed entirely. Macy and Outhouse (1924) give a résumé of investigations of cotton-seed flour as human food.

Leaves.—The leaf has dietary properties very different from the seed. The contrast between the state of nutrition which can be secured with suitable mixtures of leaf and seed, as compared with complex mixtures of seeds, was first pointed out by McCollum, Simmonds and Pitz (1916-1917). It is impossible to secure satisfactory nutrition in animals fed exclusively seeds and seed products. Alfalfa leaf was first studied because the immature leaf is available commercially as alfalfa flour.

The dietary properties of mixtures of leaf and seed are well illustrated by a series of nutritional experiments carried out with mixtures of seed 60 per cent. and alfalfa leaf 40 per cent. The seeds were wheat, maize, rolled oats, rice, pea, and navy bean. The amount of growth with mixtures of one kind of seed with one kind of leaf is much greater than can be secured with combinations of wheat, maize, oat, hemp, and millet seeds in any proportions. A mixture of these seeds can support a fair amount of growth if its inorganic deficiencies are corrected, but without mineral salt additions almost no growth is secured. Chart VII shows typical records of animals confined to diets of leaf and seed. Among the seeds studied the oat is best supplemented by alfalfa leaf. A mixture of 60 per cent. rolled oats and 40 per cent.

alfalfa leaf induces in the rat nearly normal growth. Animals grown on this diet showed moderate fertility and fair success in rearing young. They were not nourished in an optimum manner, for they fell considerably below the maximum capacity of well-nourished rats in fertility and rearing of young.

A study of leaves of other plants showed that these can be classed together as foods of similar character. They resemble each other as do seeds in their nutritive properties. The dry leaf contains two to five times as much ash as the seed, and is always especially rich in just those elements in which the seed is poorest, viz., calcium, phosphorus, sodium, and chlorine. It follows that the leaf supplements the inorganic deficiencies of the seed. The leaf contains in most cases much more vitamin A than do seeds. The protein of dry leaves varies from 8 per cent. in such fleshy leaves as cabbage, to more than 15 per cent. in alfalfa and clover, and to 30 per cent. or more in the dry leaf of the turnip. Not all the nitrogen in leaves is in the form of compounds which have nutritive value. From data available, the proteins of the leaves appear to supplement the proteins of the seeds. The leaf supplements all the deficiencies of the seed, but not in a highly satisfactory manner in every case.

Differences in Function of Leaf and Seed.—The leaves and seeds of plants show as great a difference nutritively as they do functionally. The seed is composed of germ, which in most cases forms but a small part of the seed, and a relatively large endosperm. The germ consists of living cells, which are capable of germination. The germ constitutes 1.5 per cent. of wheat. The endosperm contains large amounts of reserve materials such as protein, starch, sugars, fats, and mineral salts. These are not living matter, but its by-products. The endosperm is therefore comparable in most respects to a mixture of purified food substances. The endosperm contains relatively few vitamins as compared with the germ. The latter is in great measure composed of the protoplasmic structures necessary for metabolism. Chlorophyll enables it to utilize the energy of light; and from carbon dioxid of the air, and from water and mineral salts from the soil, it builds up protein, starch, sugar, and fat, to be used for growth or for storage in seed or tuber.

Thin Leaves More Complete Foods Than Thick Ones.—The dietary quality of leaves differ considerably. Some are thin, cellular structures which dry easily; these have little reserve food and relatively little inert cellulose. Others, such as the cabbage

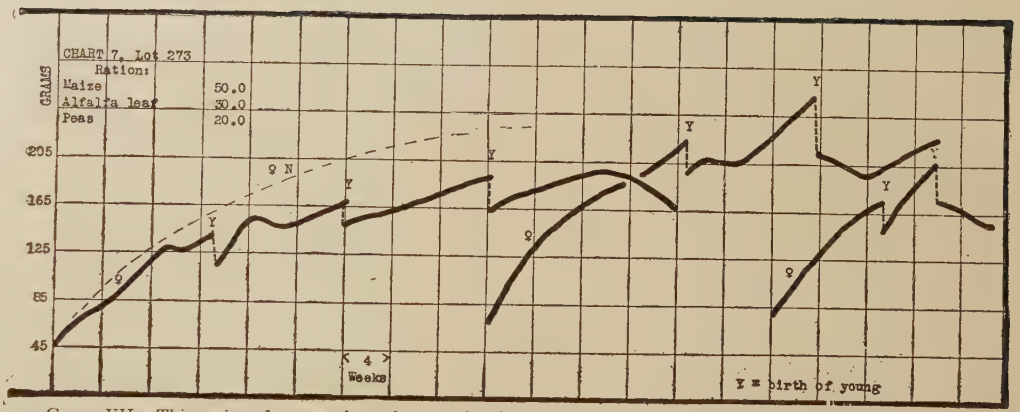


CHART VII.- This series of curves show the records of a mother, her daughter and granddaughter, all of which were restricted throughout life to a diet consisting of maize, alfalfa leaves and peas. This simple mixture makes a fairly satisfactory vegetarian diet. This mother and her offspring were not so large as other animals in the same group, but her record is presented because young of the fourth generation, a great-great-granddaughter, was successfully weaned on this diet without any noticeable deterioration in the vigor of the family. The diet was not of the best quality, for the infant mortality was high, and there was a tendency for the animals to remain undersized. It shows, however, that it is easily possible to successfully rear rats on a strictly vegetarian diet.

leaf, are modified as storage organs; like the seed endosperm they contain reserve food. Cabbage leaf also contains much cellulose which serves as supporting tissue. The dietary properties of such leaves are modified accordingly. The cellular elements are diluted by the inert tissues and by the reserve food substances in them. The less a leaf has of the function of a storage tissue, the more pronounced will be the leaf quality as a food. Fleshy leaves tend to have the dietary properties of seeds, and in this respect stand between the thin leaves and the seeds. In alfalfa, a typical thin leaf of high nutritive value as a forage plant, the content of vitamin A is greater than of B. Steenbock and Gross (1920) found 5 per cent. of immature alfalfa leaf as the sole source of vitamin A supported normal growth and rearing of some young. Lettuce, spinach and chard contain vitamin A in similar amounts, but lettuce appeared to be somewhat poorer in it than the other leaves studied.

Osborne and Mendel (1920) found alfalfa and clover leaves to surpass tomato, spinach, cabbage, turnip, carrot, beet root and potato in vitamin B content. All of these contain A, but beet contains less than other roots. This is in harmony with the data of Steenbock and Gross (1919). One gram of dried clover or alfalfa per day fed to a rat for vitamin B gave better growth than the same diet with 16 c.c. of milk. Mendel (1920) estimated that a tenth of a gram of butter fat or a tenth of a gram of dried tomato fed daily as the source of vitamin A, was sufficient to enable rats to grow to maturity and maintain them for 14 months. Osborne and Mendel (1922) studied richness of leafy vegetables in vitamin B. Asparagus, celery, and lettuce contained more B in terms of edible product than apples, pears, or the juice of grapes. Calculated as fresh vegetables, 2.2. gm. of asparagus, 11 gm. of lettuce, 10.8 to 17.2 gm. of celery, given daily as sources of vitamin B, produced excellent growth. Dandelion alone gave rather poor results, but 17.0 gm. of dandelion plus 0.2 gm. of dry yeast induced good growth.

Hume (1921) and Steenbock (1922) found white cabbage leaves inferior to green in vitamin A content. White sweet potatoes and white carrots contained little vitamin A. The tops of white carrots contained more yellow pigment and were richer in A than the roots. Green outside leaves of cabbage contained more vitamin A than inner white ones. Coward and Drummond (1921) state that etiolated seedlings and chlorophyll-free cabbage leaves do not synthesize vitamin A, whereas green leaves

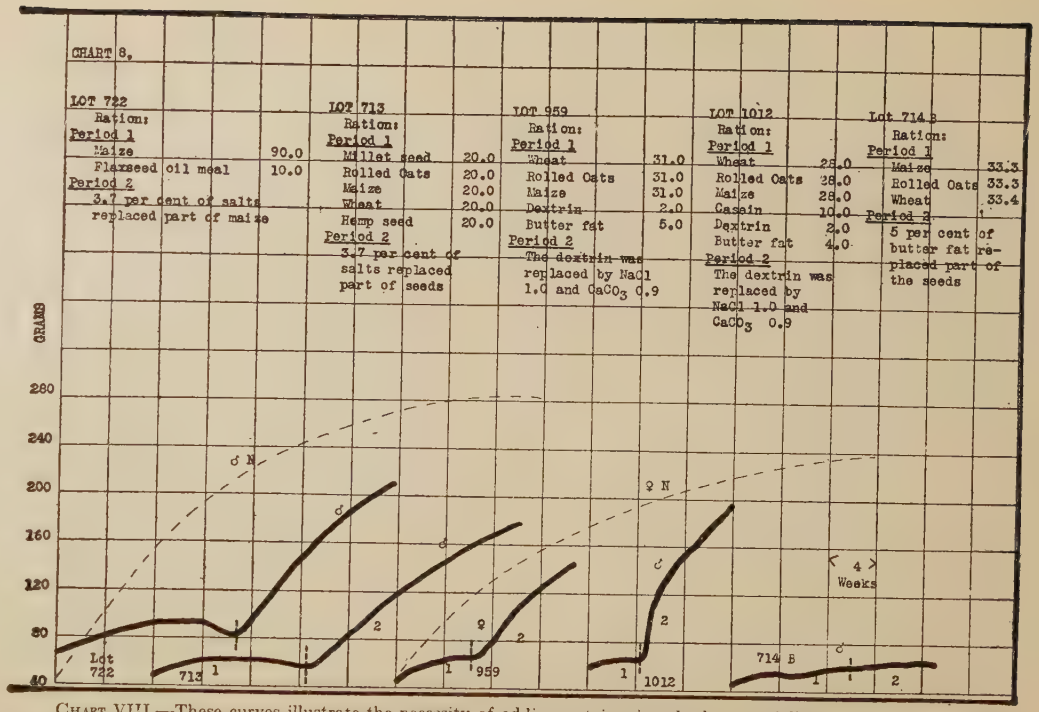


CHART VIII.—These curves illustrate the necessity of adding certain mineral salts, especially calcium salts and sodium chlorid. to mixtures of seed products. Lots 722, 713, 959 and 1012 were unable to grow in period 1, but in all cases growth was possible when salt mixtures were added. Lot 714 B made no response with growth when after a period of stunting butter fat was added to their diet. Mineral salts are the first limiting factor in preventing growth in animals fed seed mixtures. Compare with Chart VII.

do. Marine algæ containing chlorophyll synthesize vitamin A, whereas organisms containing red pigments contain little, and mushrooms devoid of pigment are almost completely lacking in it.

Tubers.—Tubers constitute, after the seeds, one of the most important classes of energy-yielding foods. The white potato is by far the most important of these in the temperate zones. In the Orient several kinds of tubers are widely used as human food. The dietary properties of the potato are what we should expect from its function as a storage organ for reserve food (McCollum, Simmonds and Parsons, 1918). The function of the potato is two-fold: the reproduction of the species, and a source of food for the potato plantlet. The "eyes" represent groups of cells which are analogous to the germ of the seed in that they give rise to the sprouts. The interior of the potato consists almost entirely of water, starch, protein, and mineral salts; it is analogous to the endosperm of the seed.

From the dietary standpoint the potato is to be classed with the seeds because it consists largely of reserve materials. The proteins of the potato and of the fleshy roots are not quite so valuable for the support of growth as those of the cereal grains when they serve as the sole source of this dietary factor. Raw potatoes are an excellent anti-scorbutic food.

Although potato is largely carbohydrate, there is evidence that its protein is of considerable value. Hindhede and co-workers (1913) in Denmark report that they subsisted for several months on a diet the protein of which was derived solely from potatoes. One man took a diet of 4½ to 9 pounds of potatoes daily for nearly 300 days with no ill effects.

Rose and Cooper found that in human subjects the nitrogen of the potato is of extraordinary value for replacing that lost through daily catabolism in the adult (1917). McCollum, Simmonds and Parsons (1918) studied this nitrogen by comparing with the protein of the cereal grains the value of the nitrogen of the potato. In growth tests in the rat potato nitrogen falls below that of cereal grains.

Kon (1928) found the protein tuberin of the potato of considerable value for growth. He and Klein (1928) working in the State School of Hygiene at Warsaw report an experiment in which a man and a woman lived 167 days in good health and in nitrogen equilibrium on a diet in which the nitrogen was derived almost solely from the potato. The man's

daily nitrogen intake averaged 5.7 gm. and the woman's 3.8 gm. The intake of potatoes was large, and was supplemented with fats and a little fruit. Digestion was excellent and the subjects felt well. It is said that deficiency diseases are uncommon where potatoes are eaten liberally.

Fleshy Roots.—These are similar to the potato in dietary properties and biological function. The roots we employ as foods are those which are highly modified as storage organs, and resemble the potato in possessing a high water and starch content and but little protein. In the sugar beet sugar in great measure replaces starch. Steenbock and Gross (1919) demonstrated that the beet resembles the seed and tuber rather than the leaf. The dasheen and sweet potato have an inorganic content which resembles that of the seeds. The composition of the ash of seeds, tubers and roots gives no promise that their inclusion in diets of seeds will correct the inorganic deficiencies of the latter. Feeding tests in which seeds and tubers or roots have been combined and supplemented with protein and butter fat (vitamin A), so that all the deficiencies of the mixture except the inorganic were made good, have confirmed this view. Denton and Kohman (1918 and 1920) studied the carrot by the biological method. Their results showed that it was possible to maintain rats without growth for some weeks on a diet composed of carrots, sodium chlorid and a calcium salt. It was necessary to add protein and vitamin A before the rat was able to grow. Morgan and Stevenson (1923) found the French artichoke to contain less vitamin B than A. Morgan (1924) found the radish to be a very poor source of vitamin A but about comparable to fresh milk or orange juice as a source of vitamin B. Morgan and Francis (1924) found fresh and dried pumpkin to be moderately good sources of vitamins A and B when tested upon mice.

Difference in Vitamin A Content of Roots.—Steenbock and Gross (1919) studied the more important fleshy roots with respect to their content of vitamin A. Their results are of unusual interest, for they showed that carrots and sweet potatoes, both of which contain much yellow pigment, are far better sources of vitamin A than are any of the roots which do not have a yellow color. Rutabaga, dasheen, red beet, parsnip, potato, mangle, and sugar beet appear to contain practically none of this substance.

Fruits.—From chemical analysis fruits appear to be comparable to the tuber and root vegetables. They contain much water, varying amounts of sugar, and relatively little protein or fat. They are more attractive to the palate than are most of the vegetables. They have mild laxative properties and tend to promote elimination by virtue of their mild diuretic qualities. This is due in part to the extra water consumption, and also to the action of certain salts of organic acids.

Fruit juices and the edible portion of fruit resemble the tuber and root vegetables and differ from the cereal grains in an important respect. An excess of basic radicles renders their ash alkaline, whereas seeds give an acid ash. Meats are also acid formers. The meat and bread portion of our diet, when oxidized in metabolism, forms an excess of acid over base-forming substances. A certain alkali reserve in the blood is essential to its capacity to carry carbon dioxide to the lungs for elimination. A common pathological condition, acidosis, exists when this reserve falls below normal. This is why eating fruits and vegetables tends to establish a proper acid-base equilibrium in the various fluids of the body.

Fruits, tubers and roots differ from leafy vegetables in containing little calcium. Osborne and Mendel (1919) found 5 gm. per day of fresh apples or pears prevented beri-beri in rats. The edible portion of orange, lime and grapefruit contain vitamin B in about the same amount as milk, and could be dried without losing their potency. Grape juice, apples and pears appear to be less potent. Tomato is still richer, having about half the potency of yeast when comparison is made on the dry basis. They found (1922) orange to contain considerable amounts of vitamins A, B and C. Stammers (1924) found that 10 c.c. of orange juice daily did not contain sufficient vitamin B for satisfactory growth in rats but protected them against beriberi. Morgan and Chaney (1924) found grapefruit, fresh and concentrated, to contain small amounts of vitamin A, but the ether extract of grapefruit peel did not contain it. Lemon juice was likewise of little value as a source of vitamin A but the ether extract of lemon peel contained a small amount of it. The juice, peel and oil of orange each contained large amounts of vitamin A. Miller (1924) found fresh and canned pineapple to be good sources of vitamins A and B.

Dutcher and Outhouse (1923) found little if any vitamin A in

any of several varieties of raisins. The B content differed in different varieties. Twenty to forty per cent of seedless raisins in the diet as a source of B sufficed to promote normal growth in rats. There was no vitamin C in market raisins.

Smith, Bergheim and Hawk (1922) report that strawberries are rich in vitamin C. Santos (1922) found that togi (sprouted mongo), okra, avocado, mongo, sweet potato leaves, and duhat (Eugenia jambolana) were relatively rich in B. Artichokes, bilimbi (Averrhoa carambola), and banana flour bud and bamboo sprouts were relatively poor in vitamin B. Mongo was found to be poor and togi when fresh relatively rich in C, but after cooking vitamin C had been destroyed. Santos also verified previous observations that in sprouting, vitamin C is increased in mongo. Ten grams of mongo as a daily supplement to a scorbutic diet failed to protect guinea pigs from scurvy, while 5 gm. of fresh togi cured pigs of this disease. Shohl (1923) also found the Jerusalem artichoke to be very poor in vitamin B.

Fresh citrus fruits, e.g., lemon, orange and lime, have long been known to prevent scurvy. Recent investigations have shown this property to be due to the presence of vitamin C. Byfield, Daniels and Loughlin (1920) found the anti-neuritic value of orange juice equal to the same volume of milk.

Banana.—The banana was studied as food for the rat by Sugiura and Benedict (1918-19), and for the banana fly by Loeb and Northrup (1917). The former found banana deficient in protein and vitamin B when it constituted about 80 per cent of the diet. Benedict and Sugiura found the banana supplemented with casein, and a source of vitamin B (yeast or carrot extract) inadequate for milk production by lactating rats. The failure lay in the quality of the milk and not in the quantity, since the mothers failed to succeed with both small and large litters. They believe a special vitamin is necessary for lactation, a view once supported by Evans.

Loeb and Northrup (1917) found sterile banana was inadequate for the nutrition of the banana fly, but banana cultured with yeast to be satisfactory. The interpretation placed upon this observation is that the yeast cell produces synthetically some substance or substances, not present in the banana, but necessary for the nutrition of the fly. Acuña (1923) says that 5 gm. of papaya (Vigna sinensis), 10 gm. of banana, and 20 gm. of papaya (Carica papaya) were needed as a supplement to a basal ration to furnish vitamin B for normal growth in rats.

Several investigators have reported that banana is a fairly good source of vitamin C.

Nuts.—Nuts, with the exception of the chestnut, are rich in protein and fat and poor in carbohydrates. The amounts of protein and fat contained in the edible portions of the more highly prized nuts are listed in the following table:

TABLE XVII

Nut	*Per cent. Protein*	*Per cent. Fat*
Almond	21.0	55.0
Beech-nut	22.0	57.0
Brazil-nut	17.0	67.0
Chestnut	6.2	5.4
Pecan	11.0	71.0
Walnut	18.5	64.0

The digestible carbohydrate of most nuts is very small. The chestnut is unique among the ordinary nuts in that it contains about 45 per cent. of starch and little fat.

The flavors of nuts are especially appetizing and they are highly prized. They are the seeds of plants and, as expected, fail to supplement cereals, legume seeds, tubers, roots or fruits, except in respect to protein. A diet composed of such combinations of food may be balanced so far as chemical analysis can show, but be defective in other respects.

Cajori (1920) estimated that nuts had about the same vitamin B value as the cereal grains. Coward and Drummond (1920) found the brazil nut, Barcelona, peanut, walnut, almond and butternut, to contain but small amounts of vitamin A. Rose and MacLeod (1922) found rats to fail even with 15 per cent. of almond oil as a source of vitamin A. Morgan and co-workers (1923) found that 20 per cent. of almond oil did not cure ophthalmia in rats. Mignon (1923) found cold pressed walnut oil when fed at 20 per cent. of the diet to prevent ophthalmia. A hot 95 per cent. alcoholic extract of blanched almonds produced sub-normal growth for 150 days when fed at 20 per cent. of the diet as the sole source of vitamin A.

The Carbohydrates.—Cane sugar, beet sugar, starch, glucose, syrup and honey are sources of carbohydrates. They are essentially sources of energy. They differ to some extent in the rate

at which they are absorbed, the simpler sugars being more readily digested and absorbed than starch. All are lacking in vitamins. Honey has been shown to be deficient in vitamins A, B and C (Dutcher, 1918, Faber, 1920, and Scheunert, 1923).

Aeptler (1922) made feeding experiments with royal jelly and found it lacked sufficient vitamin A to promote growth in young rats, but was relatively rich in vitamin B. It is suggested that the queen larva has but a small requirement for vitamin A. This vitamin is apparently derived from pollen.

Animal Tissues.—Veal, beef, pork, lamb, mutton, fish, and fowl are the commonest animal products used as human food. Animal tissues must be differentiated into two groups, viz., glandular organs, such as liver, sweet bread and kidney, and the highly specialized muscle tissue. Those organs which are the seat of metabolic activity of the type concerned with the elaboration of secretions or of intermediate or end-products of metabolism are more complete foods than are the supporting and contractile tissues. The marked differences in the properties of leaves of plants as contrasted with storage tissues have a parallel in the nutritive value of animal tissues having different functions.

Watson (1904 and 1906) restricted birds to an exclusive diet of muscle meat and observed profound pathological changes, viz., nervous disturbance and paralysis (polyneuritis), hypertrophy of the thyroid and of the parathyroids. An exclusive diet of horse muscle and of beef muscle (1906) for rats caused young ones to die and older ones to be incapable of producing and rearing young. The diets were faulty in respect to minerals and all the vitamins.

Vitamin Content of Animal Tissues.—Cooper (1912-1913) made a study of the relative values of food-stuffs for the prevention of polyneuritis in pigeons. The number of grams of fresh substance per day necessary to prevent the disease were: ox muscle, 20; ox cardiac muscle, 5; ox cerebrum, 6; ox cerebellum, 12; ox liver, 3; sheep cerebrum, 8-15; fish muscle, more than 10; egg yolk, 3; cow's milk, more than 35; cheese, more than 8. McCollum and Davis (1915) and Osborne and Mendel (1917-1918) showed that an ether extract of pig kidney or of codfish testicles contained vitamin A, and that heart muscle contains less of it than kidney.

Muscle tissues stand in marked contrast to the glandular organs. Osborne and Mendel (1917-1918) showed that beef muscle is very deficient in vitamins A, B and C, but that liver

is a good source of A and B. Meat extracts are also deficient in these respects. The proteins of muscle, liver, kidney and brain are complete as sources of amino-acids for growth when each serves as the sole source of protein. Dutcher and co-workers (1919) found raw lean beef to be deficient in vitamin C. Damon (1922) found that commercial beef extract or peptone did not contain B. Parsons (1920) found fresh liver to be rich in vitamin C.

Hoagland (1923) states that lean pork is rich in vitamin B, comparing favorably with the vitamin content of liver and kidney. Heart is reported to be richer in B than either liver or kidney. Beef contains less vitamin B than pork, and mutton stands between them. These results are not in harmony with those of others. Wright (1923) found fresh beef, mutton, lard and pork, as well as beef which had been stored two years, lamb stored three years and pork stored nine years, all comparable in vitamin B content. He concludes that storage of meat is without effect on the vitamin B content.

Animal Fats.—McCollum and Davis (1913) and Osborne and Mendel (1913) found that lard had no appreciable growth-promoting properties when added to a diet complete except for vitamin A. The latter found cod liver oil to be an excellent source of A. They found (1915) the more liquid portion of beef fat to contain this dietary factor, whereas the unfractionated fat was very poor in it. Halliburton and Drummond (1917) obtained similar results in their study of various kinds of margarines.

Daniels and Loughlin (1920) found some samples of lard to contain demonstrable amounts of vitamin A. Drummond and co-workers (1920) believe that a pig can store vitamin A when receiving it in abundance. They bring out the interesting point that the concentration of vitamin A in pig fat, even in grass-fed animals, appears to be considerably lower than in the fat derived from grass-fed cattle, and much lower than that usually found in butter. Since pigs are not ordinarily fed diets rich in this vitamin, lard is not a reliable source of it. The vitamin A content of beef fat, like that of lard, depends upon the diet of the animal. Hydrogenated fats of animal and vegetable origin and lard compounds are usually devoid of vitamin A. Whale and other marine animal fats contain it, but it is destroyed in the process of hardening. Osborne and Mendel (1921) found no essential difference in the results obtained with lard extracted

with alcohol, commercial lard, and lard rendered at comparatively low temperatures. Mallon and Clark (1922) found 30 per cent. of lard from a pig fed a diet rich in vitamin A did not contain enough to prevent xerophthalmia. Rendering probably results in oxidation of a part of vitamin A.

Soft Pork.—Ellis and Hankins (1925) have reviewed the literature relating to the production of soft pork. The difference in the firmness of the chilled carcasses of young, immature pigs and fully developed mature hogs when fed certain rations has been studied in several Agricultural Experiment Stations. Ellis and Hankins found that the change from a soft fat as found in young, immature hogs to a hard fat in older mature hogs was accompanied by an increased rate of fat deposition. The change in the composition of the body fat resulted in a decrease in the iodine number and refractive index and an increase in the melting point. These studies have been continued by Ellis and Isbell (1926). They studied the influence of the character of the ration upon the composition of the body fat of hogs. The following rations were used: (1) Brewers' rice with tankage; (2) Corn with tankage or fish meal; (3) Two parts corn meal mixed with 1 part peanut meal; (4) Rice polish with tankage; (5) Soy beans plus 2.5 per cent. of shelled corn; (6) Peanuts grazed or self-fed in dry lot; (7) Soy beans grazed; (8) Corn plus tankage plus (a) peanut oil, (b) soy bean oil, (c) cottonseed oil, (d) corn oil; (9) Peanuts followed by corn with protein supplement; (10) Soy beans followed by corn with protein supplement. They found that the percentages of saturated acids, oleic acid, and linolic acid of lard from peanut and soy bean fed hogs were similar to the content in peanut and soy bean oils. The linolic acid showed the most striking change, increasing from 1.9 per cent. in the hard fat from hogs fed brewers' rice to 30.6 per cent. in oily fat from hogs fed soy beans. Their studies show very clearly the close relationship between the diet and the kind of fat produced. The vitamin A content of these various kinds of lards was not studied. These studies are of great value since soft pork was a great economic loss.

Diet and Body Fat of Rat.—Anderson and Mendel (1926) studied the influence of the diet on the character of the fat deposited in the body of the rat. Rats fed a soy bean oil and peanut oil diet, then subjected to fat depletion through selective starvation involving 23-27 per cent. loss in body weight before being fed a hardening diet, yielded harder fats (fats lower in

iodine values) than rats not starved before being fed the carbohydrate-rich diet. In other words, through the process of starvation the soft oily fat produced from soy bean and peanut oils is largely removed, permitting the deposition of a hard fat. To obtain a fat of equal hardness from rats which were not subjected to the starvation treatment would have required a much longer period of feeding a starch-rich diet. They also found (1928) that in rats fed whole wheat and whole milk the body fat has a lower iodine value, i.e., becomes harder as the weight increases. Fifteen per cent of the caloric intake was supplied as protein from dried skim milk, which supplied 40 per cent. of the total calories of the ration. The remaining 60 per cent. was variously offered as either fat or carbohydrate. When soy bean oil, corn oil, cottonseed oil, or peanut oil was ingested, the resulting body fat resembled the food fat. When butter fat or coconut oil was consumed the body fats contained more unsaturated fatty acid than the ingested fat. Corn starch, dextrin and dextrimaltose substituted equicalorically for fat in the diet produced hard body fats. When casein or cottonseed globulin were fed to the extent of 96 per cent. of the total calories a hard fat was produced similar to that from diets rich in carbohydrate. When a particular type of soft body fat has been produced its chemical make-up can be altered by a change to the hardening ration rich in carbohydrate.

Vegetable Oils.—Numerous experiments have shown that vegetable oils such as olive oil, cottonseed oil, maize oil, soy bean oil, coconut oil, sesame oil, etc., are essentially lacking in vitamin A. On the other hand, a mixture of substances extracted by fat solvents from alfalfa leaves was shown by Osborne and Mendel to contain some A. While fat solvents may extract small amounts of the vitamin, fats extracted from vitamin-rich foods by this means are not effective sources of vitamin A. Halliburton and Drummond (1917) tested coconut oil and nut butters and found them practically devoid of vitamin A. Jansen (1919) confirmed the observation that coconut oil contains little, if any, vitamin A.

Stammers (1924) compared crude and rancid maize oil with the refined product. They contained vitamin A in amounts comparable to palm oil and also winter butter fat. He believes that the vitamin content of vegetable oils depends on the processes of refining. This view is confirmed by Drummond and Coward (1920).

Fish Oils.—Livers of fish do not serve as a storehouse of carbohydrate (glycogen) as do livers of mammals, but are loaded with fat when the fish is in prime condition. All liver oils from fishes are excellent sources of vitamin A. Fish oils so far examined contain vitamin D. Sekine (1920) found the oil of the meat of Shya-chi-uo and that from the abdominal muscles of tuna to contain liberal amounts of A.

The Content of Vitamins A and D in Cod Liver Oil.—Clare and Soames (1928) found there is no parallelism or interrelationship between the content of vitamins A and D in a number of samples of cod liver oil. Some oils which are rich in A were poor in D, and others the reverse. These findings are all of significance at the present time since some commercial firms have demonstrated a satisfactory A content by animal tests and without examination for D have extolled the potency of such oils for preventing or curing rickets in children.

Milk.—Milk is not a complete food when taken over a long period as the sole source of nutriment, but is the one food for which there is no effective substitute.

Milk is a very effective food for supplementing the deficiencies of other substances such as cereal grains, tubers, and fleshy roots (Lane-Clayton, 1916; McCollum, Simmonds and Parsons, 1921). It is rich in calcium and phosphorus, whereas many vegetable foods are poor in these elements. Calcium and phosphorus are prominent constituents of bone. There are but two classes of foods which are rich in calcium, the principal element in lime; these are the milk of animals and the leafy vegetables. All other foods fall below the calcium requirements of man and animals. The body is very sensitive to deviations from the optimum concentration of calcium in the food. Serious damage results to the bone structures if the diet is not properly constituted.

Dietary Properties of Milk.—Milk contains about 87 per cent. of water and 13 per cent. of dissolved substances consisting for the most part of proteins, milk sugar and fats. It is too bulky to be satisfactory as the sole food for the adult. Its value is as a supplement for other foods to correct their deficiencies. Most animal and vegetable foods are singly or collectively faulty in sufficient degree to interfere with the physical development of the young and to lead to early deterioration of the adult. A most important consideration to be kept in mind is, therefore, the selection of food so as to compensate for the deficiencies of one article by those of another and thus make a properly bal-

anced dietary. For this purpose milk is the ideal food. Milk and the leafy vegetables are the only foods so constituted as to make good the deficiencies of cereal grains, legume seeds, tubers, roots, and muscle meats. For this reason McCollum (1918) proposed that these foods should be designated "protective foods" in order to give them the prominence they deserve. Fresh fruits or certain raw vegetables should be included in the diet regularly in order to insure a sufficient amount of vitamin C.

The supplementary value of milk for other foods depends upon its unique calcium content, on the quality of its proteins, and its fat. The proteins of muscle, liver and kidney are more valuable for enhancing the proteins of cereal and legume seeds than are the proteins of milk. It is only in this respect, however, that the animal tissues surpass milk (McCollum, Simmonds and Parsons, 1921).

Milk varies considerably in its vitamin content. Hopkins (1912) reported that 2 to 3 c.c. of milk in the diet of a young rat supplied sufficient amounts of vitamins A and B for a small amount of growth. On the other hand Osborne and Mendel (1918, 1920, 1922) found 10 to 16 c.c. of milk necessary to provide enough vitamin B for a rat. Johnston (1921) and Dutcher and co-workers (1920-1921) sustain the contention of Osborne and Mendel that the small additions of milk made by Hopkins are inadequate to supply vitamin B for the rat. Hopkins (1920) reiterated the statement that 2 to 3 c.c. are sufficient. Hopkins's experiments were very short, whereas those of the other investigators covered several weeks or months. This accounts, we believe, for the differences in their results.

McCollum, Simmonds and Pitz (1916) demonstrated the inability of little rats to grow when nursed by mothers whose diets were deficient in either A or B or both vitamins. They demonstrated that the lactating female cannot put the vitamins into her milk unless they are furnished by her food. Kennedy and Dutcher (1922) found 10 c.c. per day of summer or winter milk adequate to furnish A and B for the rat provided the ration of the cow carries each in adequate amounts. Winter farm milk was deficient in vitamins. The increase of the vitamin A content of the milk produced by feeding cod liver oil to cows was not accompanied by a rise of lipochrome pigments as is the case when cows are fed on grass.

Common experience shows that the young of all mammals grow well for a period during infancy when milk constitutes the sole

food. Experiments have shown, however, that milk is not a complete food over long periods, and that it should be supplemented with other foods capable of supplying whatever nutrients are deficient in milk. Young rats fed fresh milk alone make good initial growth but some time between the 50th and the 100th day of life, especially in females, there is a decided retardation. There is no reproduction. On dry milk alone rats make much better growth but do not produce young. The addition of small amounts of yeast, wheat germ, butter fat, etc., which are especially rich in vitamins, improve somewhat the state of nutrition, as does the provision of an iron salt,—but none of these serve to make milk a complete food. It has long been known that milk is deficient in iron, and many have supposed that this is its only deficiency. For some years it has been known that this is not the case. Recently, Hart, Steenbock and their co-workers (1928) have discovered that copper is an indispensable element in nutrition and that deprivation of this element interferes with iron assimilation. Milk, they found, is deficient in copper as well as iron, and hence it seems probable that lack of copper was in many of the experimental studies on milk in the past the limiting factor which caused subnormal development and sterility.

Sherman and co-workers (1921-1922) described the results of feeding combinations of bread and milk, bread and apple, bread and turnip, and milk and wheat. Diets in which milk furnishes from one-third to two-thirds of the total solids were superior to diets similarly constituted but with smaller percentages of milk. Rose, MacLeod and Bisbey (1923-1925) studied the maintenance value of milk, bread and milk, and meat and soy bean curd in human nutrition. Their basal diet contained arrow-root starch, dextrimaltose, lactose, butter fat, and apple. Young women were subjects in experiments covering 12 to 15 days. In a 12 day period the nitrogen stored was 2.12 gm. with soy beans, 4.33 with meat, 41.43 with milk and 30.37 with bread and milk.

Fats of Milk Good Source of Vitamin A.—The fats of milk are the most important source of vitamin A in American and European diets. Vitamin A is essential for both growth and maintenance; this will be considered more in detail later. Hart (personal communication) informs us that probably over 90 per cent. of the vitamin A in whole milk is removed in the skimming process.

Smith (1922) found vitamins A and B present in commercial samples of ice cream.

Ghose (1922) found Indian ghee (butter fat made from buffalo milk) as rich in vitamin A as butter.

Widmark (1924) states that the export of butter from Denmark is the cause of the prevalence of ophthalmia. The population finds in margarine a substitute for butter. A few years ago margarine was prepared from animal fats which contained a little vitamin A, but during recent years there has been an increasing use of vegetable margarine, since this is cheaper. The latter is almost devoid of vitamin A.

Effect of Pasteurization on Vitamin, Calcium and Phosphorus Content.—Milk is pasteurized to destroy certain disease-producing organisms which it may contain. It is heated to 143° to 145° F for 30 minutes in the commercial process. Milk loses its vitamin C content in great measure in pasteurization but neither A nor B is appreciably affected.

In pasteurization the calcium salts of milk are rendered more or less insoluble, depending on the length of time the milk is heated. In this insoluble form they may be lost through adhering to the container. This has long been known because of the formation in the pasteurization apparatus of a deposit called "milk stone." Daniels and Loughlin (1920) and Daniels and Stearns (1924) have studied this problem. They found that when special care was taken to include the insoluble material in evaporated milk, results comparable to those on raw and quickly boiled milk were obtained. Rats fed super-heated milk with di-calcium phosphate addition made normal gains. They believe the inferior growth of rats on long heat-treated milk and super-heated milk to be due wholly to the readjustments of the inorganic complexes, rendering assimilation difficult. They regard it as probable that a baby fed pasteurized milk over a long period receives too little calcium for growth needs. Magee and Glennie (1928) state that vigorous boiling of milk may bring about a loss of its small content of iodine. In their tests heat caused the disappearance by volatilization of 20 per cent. or more according to the duration of heating, of the total iodine of separated milk. These observations emphasize the importance of adding fruits and vegetables to a child's diet at an early age.

Milk Frequently Soured to Preserve It.—Milk is not only the most valuable human food but is likewise an unsurpassed nutrient medium for the growth of bacteria, hence it is liable

to spoil unless properly handled. Pastoral peoples living under primitive conditions promptly sour milk by permitting a vigorous growth in it of lactic acid-producing bacteria. This protects it against proteolytic organisms which produce unwholesome decomposition products. The rise of acidity is so rapid that all other organisms and certain yeasts are either killed or their growth inhibited. Sour milk is a wholesome food, and is used in enormous quantities in Asia, Arabia, the Balkan States, Northern Africa, and in the grazing sections of Abyssinia. Wherever it is used in liberal amounts as human food the people are of exceptional physical perfection. This will be discussed later (Chapter XXVII).

Blackham (1920) examined the milk supply in the tropics from the cow and buffalo. He found it to be both highly adulterated and highly contaminated. Buffalo milk contains about twice as much fat as does cow milk. In several countries of Europe and in India the bacterial content of milk is often greater than that of sewage. The solution of the milk problem in India is the development of some substitute for fresh milk such as milk powder which can be kept indefinitely in a dry state.

The number of bacteria in milk depends upon the temperature, age and amount of dirt which contaminates it. In America it is the custom to market liquid milk pasteurized. In order that this may be done in a satisfactory manner great care must be exercised in respect to cleanliness of milkers, or cows and of utensils. Pasteurization will make dirty milk safe but it will not make it good milk. Refrigeration must be effective and delivery prompt. There has been steady progress in the supervision of the milk supply of cities in recent years and America has the most wholesome milk supply in the world. *All milk should be pasteurized.* This prevents the spread of typhoid fever, scarlet fever, septic sore throat, tuberculosis, and other milk-borne diseases.

Bacteria in milk are related to infant mortality. Children fed clean milk with few bacteria have a lower death rate than those who take milk with high bacterial counts. Organisms which are relatively harmless to adults may cause disease in infants and young children.

Certified Milk.—Certified milk is generally sold raw. It is the highest quality of milk possible of production and is about as safe as any human institution is likely to be, yet there is a certain hazard in the drinking of unheated certified milk. A considerable number of epidemics have been unmistakably traced

to its use. The reason for this is that the danger lies in the human element in certified milk production and distribution. Experience has shown that now and again a person having a transmissible disease will contaminate the certified milk and so start an epidemic.

City Milk Should be Rigidly Inspected.—The desirability of thorough control of a city milk supply through the agency of the health department has been fully justified by results. Bacterial counts of market milk should be made regularly. They are an index of the care exercised in keeping healthy cows, in exercising cleanliness on the farm, of adequate refrigeration and promptness of delivery. Dairy inspection has come to be a well-recognized method of insuring a wholesome milk supply.

Canned Milks.—Canned milks comprise evaporated milk and condensed or sweetened evaporated milks. In the heating process there is a tendency for the calcium of the milk to separate as insoluble calcium phosphate, and the anti-scorbutic properties of the product are lost. The experiments of Daniels have shown that rats utilize the calcium in such milks. Evaporated milks are sterile bacteriologically, whereas condensed milks are preserved in great measure by reason of the high sugar content (about 40 per cent.). Evaporated milks, diluted and acidified with lactic acid, have been much used in infant feeding during the last two years, and appear to be safer than condensed milks for this purpose. The convenience of canned milks assures their continuance as the household supply where fresh milk is not readily available.

Milk Powders.—These are of two general kinds: those made by the drum process in which the milk is dried on a steam-heated revolving drum from which it is removed by a knife edge and subsequently ground to a powder; and those made by spraying evaporated milk into a well-ventilated chamber from which a blast of warm air removes the moisture almost instantaneously. Very high quality powders are now prepared and find an enormous outlet, especially in the confectionery and baking industries. Some milk powders are said to retain a considerable amount of anti-scorbutic value. Milk powders are a wholesome and convenient source of the valuable constituents of milk. Experiments by several pediatricians have shown that they may be successfully used in infant feeding when necessary.

Cheese.—One of the most concentrated of milk products is cheese. There are many varieties, depending upon the process

of manufacture. Texture and flavor vary with the different kinds. Cheese represents essentially the casein of milk together with a part of its lactalbumin and whey constituents. Since much of the calcium of milk remains with the curd in cheese manufacture, cheese is one of our calcium-rich foods. Cheeses are very rich foods and few people can eat much of them without digestive disturbances. They should be regarded as supplemental foods and used to confer flavor upon otherwise rather bland dishes.

Morgan (1926) studied the vitamin A content of three types of cheeses: American Cheddar, Limburger, and Swiss (Emmenthaler). One-half gram daily of California cream cheese (Cheddar type) or of Limberger (New York) fed to young rats suffering from A deficiency caused rapid recovery from sore eyes and resumption of growth. Under similar experimental conditions one-half gram doses of Swiss cheese (from Switzerland) neither cured ophthalmia nor restored growth. With one gram doses growth was normal but the eye disease persisted to some extent.

Iron Content of Foods.—Sherman has estimated that the average American dietaries contain from 14-20 mg. of iron per day, and that they do not, therefore, furnish a sufficient surplus of iron to justify leaving the supply of this element to chance. He suggests that foods should be selected with reference to the kinds and amounts of iron they contain. Older analyses for iron were in many cases inaccurate. The following tables give the latest and most accurate values for this element in a number of important foods.

TABLE XVIII

Iron Content of Fresh Beef Tissues*

Kind of Tissue	*Per Cent. Iron in dry matter*	*Per Cent. Iron in fresh tissue*
Bone marrow	0.0010	0.0009
Brain	0.0134	0.0023
Heart	0.0236	0.0048
Kidney	0.0292	0.0055
Liver	0.0294	0.0083
Lung	0.0621	0.0122
Muscle, round steak	0.0164	0.0041
Muscle, T-bone steak	0.0141	0.0037
Pancreas	0.0299	0.0060
Spleen	0.0383	0.0089

* Elvehjem, C. A., and Peterson, W. L.: "The Iron Content of Animal Tissues. Jour. Biol. Chem., 1927, vol. 74, p. 433.

TABLE XIX

IRON CONTENT OF MEAT SAMPLES *

Sample No.	*Kind of Meat*	*Iron Per cent.*	*Mg. iron per 100 gm. of protein*
105	Bacon cured	0.0013	12.5
107	Ham cured	0.0014	6.6
114	Pork shoulder	0.0015	8.2
108	Pork hind quarter	0.0015	7.6
112	Lamb shoulder	0.0016	8.5
113	Lamb hind quarter	0.0016	7.9
102	Beef rib	0.0024	11.9
100	Beef round	0.0025	11.3
101	Beef loin	0.0025	12.2
103	Beef chuck	0.0025	12.6
109	Veal fore quarter	0.0023	10.6
110	Veal hind	0.0027	12.2
116	Veal kidney	0.0040	24.5
111	Beef heart	0.0044	27.2
117	Beef brain	0.0053	50.5
104	Beef liver	0.0082	39.7
115	Beef spleen	0.0138	72.9
106	Beef kidney	0.0188	116.6
118	Beef blood	0.0444	247.5

* Forbes, E. B., and Swift, R. W.: The Iron Content of Meats. Jour. Biol. Chem., 1926, vol. 67, 517.

TABLE XX

IRON CONTENT OF CERTAIN REPRESENTATIVE FOODS *

Class	*Sample*	*Iron in Sample (dry basis)*
Cereals	Wheat flour	0.00091
Fish	Salmon	0.00344
Fruits	Peaches	0.00285
Nuts	Almonds	0.00423
Vegetables	Potatoes	0.00386
	Head lettuce	0.01260

* Peterson, W. H., and Elvehjem, C. A.: The Iron Content of Plant and Animal Foods. Jour. Biol. Chem., 1928, vol. 78, p. 215.

Manganese Content of Foods.—Lindow and Peterson (1927) report the manganese content of 84 samples of food. The figures range from none in fish to 0.0216 per cent. in northern grown lettuce. Different samples of the same food vary in manganese content. A sample of lettuce grown in the north contained twice as much manganese as a sample grown in the south. In 22 samples of cabbage the manganese content ranged from 0.00052 to 0.00159 per cent.

Copper Content of Foods.—Guerithault (1927) has analyzed many plants for their copper content. The following amounts of copper per kilogram of fresh material were found: Sorrel leaves 2.4 mg., spinach leaves 1.8, lettuce leaves 2.0, carrot root 2.2, turnip root 3.0, radish root 3.8, leek 2.5, watercress 1.5, salsify root 3.2, celery 2.0, onions 2.9, beets 3.2, potato 1.9, cucumber 2.3, French beans 1.8, pumpkin 1.1, tomato 2.0, cauliflower 2.0, white headed cabbage 1.8, asparagus 7.0, pears 2.2, apples, cherries, grapes about 1.4 each, oranges 3.1, olives, bananas and dates about 2.4 each, chestnuts 3.2, hazelnuts and sweet almonds about 13.0, lentils 6.6, kidney bean 10.0, peas 7.2, soy bean 0.9, wheat 7.2, rye 7.5, barley 6.5, oats 17.1, maize 6.8, polished rice 6.4, mushroom 2.2, cow milk 0.6, beef 2.1, veal 1.4, yolk of hen egg 1.8, white of egg 0.0, wheat flour 2.0, wheat germ 48.0. The human organs are classed as follows in decreasing order of copper content: liver, pancreas, hair, gray matter of brain, spleen, bones, muscles, blood, and skin.

King and Etzel (1927) found the copper content of jellies and other fruit products to be from 2 to 110.0 parts per million, and in milk from 0.40 to 0.72 parts per million.

Copper, Manganese and Zinc in Foods.—McHargue (1925) has given much attention to the significance of copper, manganese and zinc in forage crops and foods. Fertile soils contain small quantities of these elements in the leaves, pericarps and germs of the seed. When corn, wheat and rice are highly milled the resulting corn meal and flour and the polished rice are deprived of the greater amounts of copper, manganese and zinc. He states that some depleted soils may require the addition of copper, manganese and zinc in order to restore and maintain fertility.

McHargue (1927) also found that the soils of the blue grass region of Kentucky are richer in copper, manganese, zinc, nickel and cobalt than are soils from other parts of the state. Small quantities of copper in addition to the manganese had an additional beneficial effect on plant growth. The fact that most of the manganese, cobalt and zinc in the seed is present in the germ, which contains most of the vitamin content, suggests that the plant uses these elements in its physiological processes.

Zinc Content of Foods.—Analyses of the blood show that there is constantly present about one-seventh as much zinc as calcium. No one questions the dispensability of calcium as a food principle, but it is not demonstrated whether zinc is neces-

sary. Hubbell and Mendel (1927) report the zinc content of several foods in mgms. per kilo as follows: Peas 51.7, carrots 43.1, beets 38.7, cabbage 17.8, milk 32.1, egg yolk 42.9, oysters 412.2, clams 22.1, lettuce 8.9, corn starch 21, commercial casein 309, commercial egg white 21, Crisco 10, yeast powder 14, cod liver oil 9, purified casein 4, washed corn starch and purified egg white 0.0.

Nickel and Cobalt Content of Tissues.—Bertrand and Mâcheboeuf (1925) found that in human tissues nickel ranged from 0.09 mg. in the liver to 0.022 in the brain, per kg. of fresh tissue. The content in muscle, fat, stomach, and uterus was uncertain. Cobalt was often present. They also found (1926) that nickel and cobalt are relatively abundant in the pancreas as compared with other organs. These elements pass into the insulin preparations so that the latter contain much larger proportions than the average organ. They (1927) studied the importance of nickel and cobalt in physiology. They fed a ration of starch, lactose, casein, etc., including iron and zinc, but no nickel or cobalt. Fourteen mice survived an average of 19.7 days. Fifteen other mice fed the same ration plus 2.5 mgm. of nickel chloride and 1 mgm. of cobalt sulphate survived on an average of 23 days.

Mineral Losses During Cooking.—Peterson and Hoppert (1925) studied the loss of mineral and other constituents of vegetables in various methods of cooking, viz., steaming, pressure cooking, boiling with moderate amounts of water, and boiling with double the quantity of water. The loss ranged from zero for calcium in steamed spinach to 76 per cent. magnesium in boiled cabbage. The approximate average losses for solids and crude protein were 15 per cent. by steaming and pressure cooking, 30 per cent. by boiling in a moderate amount of water, and 40 per cent. in double quantity. For calcium the losses were 10 per cent. for steam and pressure cooking, and 20 and 30 per cent. respectively for the two boiling methods. Average losses for phosphorus and iron were the same as those for magnesium except in the case of boiling procedures, where the figures were 5 to 10 per cent. higher. Cabbage, celery, beet greens, and onions showed notably large losses, especially of minerals in the case of the three last named. Spinach retains all its calcium when cooked.

Eggs.—Eggs contain everything that is necessary for the growth and maintenance of the body, but are poor in calcium,

and are unbalanced with respect to certain other principles. Egg yolk is a good source of iron. Eggs are very rich in fats and related substances, but are nearly lacking in carbohydrates. They favor putrefactive processes in the alimentary tract.

McCollum and Davis (1913) found the fats of eggs fully comparable with butter fat as a source of vitamin A. The proteins of egg are of high biological value. The developing chick absorbs considerable lime from the shell, and in this way makes good the shortage of calcium in the organic content. Since we remove the shell when we eat eggs we eat the part which is deficient in lime.

Osborne and Mendel (1923) found egg yolk poor in vitamin B. One-half gram of fresh egg yolk per day was necessary for growth in a 100 gm. rat. They estimate that a quart of whole milk is equivalent to 6 or 7 whole eggs in vitamin B. The high content of vitamin B stored in the germ of seeds and in eggs of birds and fishes is a provision for the needs of the young.

Eggs have an important place in enhancing the diet. They should be eaten with a diet rich in carbohydrates to discourage the tendency to putrefaction in the intestine. They should be used to confer palatability on other foods rather than as a prominent constituent of the food supply.

Sea Foods.—Oysters, crabs and clams assume a new importance in the light of the nutrition studies which have shown clearly the inferiority of muscle meats as compared with the secretory organs and tissues which are concerned with the transformation of foods to end-products of metabolism, i.e., the glandular organs. In clams, oysters and crabs, we eat the entire animal except the shell. Their consumption insures that all the uncharacterized food substances will be present in the diet, and makes these supplementary in the sense that they are likely to add to the diet some principles which it contains in amounts below the optimum. Sea foods are of special significance in that their iodine content is higher than that of land plants or the flesh of land animals.

Randoin (1923) detected vitamin C in raw oysters. Shellfish doubtless played a most important rôle in the nutrition of early man. Numerous shellheaps of "kitchen middens" of enormous proportions occur on the Atlantic coasts of America and Europe. These were the sites of human habitations over long periods and indicate that shore dwellers depended in great measure on shell-

fish for their sustenance. Their type of diet represented a peculiar kind of carnivorous feeding which was very satisfactory.

Jones, Nelson, Murphy and Devine (1928) have recently reported further on the vitamin content of various sea foods. They found that while oysters were rich in the vitamin B complex, neither hard nor soft clams contained appreciable amounts of it. Oysters were also much richer in vitamin A than clams, but clams were richer in vitamin D than oysters.

The demonstration in recent years of the unique quality of fish oils as a source of vitamin D suggests the importance, hitherto unsuspected, of fish in the diet. Cod liver oils have been reported to contain about 0.001 per cent. of iodine; this is about one thousand times more than is present in food-stuffs such as cereals. The percentage of fat in the different species varies greatly. Salmon, shad, herring, butterfish and mackerel are well-known fatty fish, but cod, haddock, cusk, pollack and flounder are lean fish. The fatness of fish often varies greatly with the seasons. Salmon and shad, which are fat before spawning, become lean immediately afterwards (Tressler, 1923). Drummond (1918-1919) found fish proteins in the diet essentially the equivalent of lean meats from mammals. Suzuki (1919) and collaborators reported that 10 per cent. of the muscle proteins of any of the marine animals was sufficient to promote growth in rats and that the nutritive value of all of the proteins was about the same.

Kik and McCollum (1928) have recently reported on the nutritive value of the proteins of haddock and herring. They found that haddock and herring proteins at 9 and 15 per cent. levels in the diets, which were otherwise adequate, were of sufficiently good quality to promote growth and well-being of the rat over an extended period. Herring proteins seemed to be somewhat superior to those of haddock in this respect.

The supplementary value of herring proteins compared favorably with those of steak, liver and kidney for the cereal grains. It was somewhat superior to haddock in this respect. Neither of these two fish supplemented the proteins of the legume seeds to an appreciable extent. The oils from these two fish contain vitamins A and D; the content of vitamin B was low.

Vitamin Content of Foods.—Much effort has been expended in recent years to determine the vitamin content of many foods. Different samples of cereal grains differ considerably in their content of vitamin B. Steenbock's observations on the differences in vitamin A content of different samples of maize support

the view that general statements as to the relative values of many of our common foods with respect to the vitamin content are all that are justifiable. The fact that we can readily prepare diets from ordinary wholesome foods which will contain several times the minimum amount of vitamins on which apparently normal nutrition can be maintained over considerable periods, tends to render academic the discussion of the advisability of taking concentrated commercial preparations of the vitamins. Investigators are agreed that there is no need for commercial vitamin preparations.

An examination of this chapter reveals that there are but two classes of calcium-rich foods, viz., milk of animals and the leaves of plants. It has been shown that foods which have the same functions have in a general way the same dietary properties. Thus the tuber and root vegetables are more or less similar in their nutrient properties, with respect to inorganic elements, vitamin content, and energy values. Their protein content is low. All cereal grains are comparable in their dietary qualities, but differ in minor degrees. The special value of fruits lies in their suitability in most cases for providing raw food of antiscorbutic value. With the dietary properties of the different food-stuffs understood as they are at present it is easily possible to make combinations which will provide highly satisfactory diets.

CHAPTER X

THE VEGETARIAN DIET

Vegetarianism Generally Practiced as Dietary Fad.— Dietetic theorists have often advocated the adoption of a fleshless diet. The proponents of this system have broken up into schools, the adherents of which differ in the fervor with which they espouse their cause. The most extreme of these, the enthusiasts of the fruit and nut diet, have been studied by Jaffa 1901-1902). They experience esthetic pleasure in the thought that their foods grow and ripen away from contact with the soil and suggest, therefore, the purity and fragrance of orchard and of forest, the poetic joy of summer and the inspiration of the rural landscape. A second group adopts the vegetarian diet because of an abhorrence of the idea of taking animal life for the sake of food. These two classes of vegetarians regard flesh eating as unwholesome as well as unethical, and defend the view that meat eating tends to moral debasement and physical degeneration. The third group, the lacto-ovo-vegetarians, who permit the use of milk and eggs along with a diet otherwise vegetarian, is less extreme in its ardor for ethical considerations, and adheres to its tenets because of conviction that it makes for physical efficiency in a greater degree than does the consumption of meat.

Faddists Discredit Themselves by Their Philosophy.— While some of the arguments offered in sober earnestness in support of abstinence from flesh foods are suggestive of mental invalidism, others demand serious consideration and an answer based upon scientific understanding. In the former category we may place the assertion that the anthropoid apes, regarded as cognates of our ancestors, live on fruits, nuts and cereals, and that this fact proves that such a diet is especially suited to the nutrition of man. One may well argue that if a change to meat eating in the era of the cave man has enabled him to develop mentally into what man is to-day, this change in dietary habits may have been indeed a great piece of good fortune. The low development of vegetable-eating animals weakens the force of

the argument. It may be suggested that it is no more advisable to permit the apes to be our guides in dietetics than in certain other ways of living. It is, however, well known that the apes eat insects, worms, eggs, small birds and such other animals as they are able to capture, and also the leaves of certain plants, as well as nuts and fruits.

There is little comfort for the vegetarian enthusiast in the comparison of the anatomical structure of the alimentary tract of man with that of animals whose diets are of different types. The dentition, the size and shape of the stomach, intestine and cecum of man clearly indicate suitability for a mixed diet. To the esthetic argument the meat eater may reply that he includes sufficient fruits, nuts and cereals in his diet to permit him to be inspired by the beauties of Nature. To the statement that a refined person cannot eat meat until it is cooked, and its flavor changed so as to disguise its origin, the reply may well be made that many fruits and vegetables are not at all appetizing until cooked and so flavored as to increase their palatability. The argument which was at one time considered to be irrefutable and to be of sufficient force to condemn meat eating rested on the content of uric acid forming substances (purines) in flesh foods. Chemical studies have shown that there are very nearly comparable amounts of purines in many vegetable foods, such as lentils, oatmeal, beans, asparagus, and notably in tea and in coffee.

Meat Eating Does Not Deaden Moral Faculties.—A stock argument of radical vegetarians is that meat eating deadens the moral and intellectual faculties. One who reflects that Moses, David, Solomon and Jesus ate flesh foods cannot be deeply impressed by it. Likewise the statement that animals living on a vegetarian diet are strong and tractable, while carnivorous animals are ferocious does not stand inquiry. The average bull or belligerent billy goat can strike terror into the heart of man with less provocation than is necessary to render dangerous a lion or a wolf. The gentle and happy disposition of the Eskimo on his carnivorous diet has frequently been commented upon.

A Valid Argument Against Meat Eating.—The more valid arguments concerning the use of meat as contrasted with the fleshless dietary regimen are based upon the view that meat is unwholesome and that it contains waste products having poisonous properties. This view is upheld by experimental results. Professor Fisher (1907) of Yale conducted experiments with

flesh abstainers and flesh eaters, and found the former possessed of greater endurance than the latter. The tests involved holding the arms outstretched, knee bending and other exercises. The flesh abstainers were three to six times as capable of endurance as were the flesh eaters. Similar results have been reported by Kellogg (1907 and 1909).

It would be unprofitable to review the old debate concerning the relative merits of meat eating and meat abstinence. There is no conclusive evidence upon which the question can be decided. Those not convinced by the endurance tests could argue that the vegetarians were enthusiastic exponents of a fad which they exerted themselves to the utmost to defend, whereas the meat eaters were not so determined to justify their practice. There were factors not then appreciated which are of paramount importance in considering the vegetarian diet. These are made clear by certain experimental work which has a direct bearing on the subject.

Slonaker's Study of Vegetarian Diet.—The most elaborate attempt to test the merits of a strictly vegetarian as contrasted with an omnivorous type of diet was made by Slonaker (1912). He fed a group of rats 23 vegetable foods, allowing free choice within limits. A similar group was fed the same foods of vegetable origin supplemented with moderate quantities of animal food. Several foods, raw or prepared, were offered at a time. No record of food consumption or of the amounts of the different foods eaten was kept. Accordingly, the results can be examined critically only in a limited way. They are, however, of interest to show how far instinct fails to guide an animal in the selection of food. Slonaker's list of foods included nearly everything which a vegetarian in Southern California would be likely to have on his table, including seeds, the milling products of seeds, leafy vegetables, fruits, tubers, and starchy roots.

The vegetarian rats grew fairly well for a time, but became stunted when they reached a weight of about 60 per cent. of the adult size, after which they never increased in weight. The omnivorous controls grew to normal adult size. The vegetarians lived on the average 555 days, whereas the omnivora averaged 1,020 days. The vegetarians grew to about half the size and lived half as long as did their fellows receiving animal food. Slonaker concluded that a strictly vegetarian diet was not suitable for the nutrition of an omnivorous animal. He could not interpret the cause of failure.

Even with Wide Variety Vegetarianism Leads to Disaster.—Slonaker's experiments were described in 1912. At this time McCollum and Davis had discovered the differences in the growth-promoting power of different fats which established the existence of vitamin A. It seemed to them that the most probable explanation of the results of Slonaker was the absence or shortage in his vegetarian diet of the dietary essential furnished so abundantly by certain animal fats, which later was designated vitamin A. A low protein intake suggested another cause for Slonaker's failure. In 1914, with these ideas in mind, they tried an experiment similar to Slonaker's, but modified it so as to give the animals a much higher protein intake than his animals probably took, and an added supply of vitamin A.

McCollum and Davis allowed their rats a choice among the following foods: wheat, maize, rye, oats (rolled), wheat germ, maize gluten, wheat gluten, flaxseed oil meal, green clover, green alfalfa leaves, onions, peanuts, and cooked navy beans and peas. With the exception of the last two the foods were uncooked. In this list there are several vegetable foods with unusually high protein content: Maize gluten, a by-product of starch manufacture, contains about 25 per cent.; wheat gluten, prepared by washing ground wheat free from starch, about 85 per cent.; flaxseed oil meal 30 per cent., and wheat germ 30-35 per cent. Animals grow well on many diets containing 9 to 10 per cent. of protein. It seemed that with this variety of proteins to select from, one possible cause of failure in Slonaker's experiments, viz., too low a protein intake, would be avoided. At that time McCollum and Davis had not discovered that the leaf of the plant is a moderately good source of vitamin A. It was assumed that when leaves and so many different kinds of seeds and seed products were supplied, there could be little doubt that everything a herbivorous animal requires was present in the food.

The rats fed this wide variety of vegetable foods, containing liberal amounts of protein, duplicated in all respects the results of Slonaker. They grew at about half the normal rate for the first few weeks, then became permanently stunted. The addition of butter fat to the diet of some failed to benefit them to a noticeable degree. The question as to why rats did not thrive on such strictly vegetarian food mixtures was not answered by these experiments. It was, however, soon learned wherein lay the cause of failure.

Successful Nutrition on Strictly Vegetarian Diet.—McCollum, Simmonds and Pitz (1916 and 1917) fed rats a monotonous diet of maize 50, alfalfa leaf (dry) 30, and cooked, dried peas 20 per cent. The ingredients were ground together so that these proportions were eaten. The diet induced growth at approximately the normal rate, and reproduction and rearing of a considerable number of young. The young grew to adult size and were successful in rearing a considerable number of offspring (see Chart VII). Without tasting, after weaning, anything other than this monotonous mixture, this family of rats remained approximately normal in vigor and successfully weaned the young of the fourth generation with no apparent diminution in vitality. The experiment was then discontinued.

Their next series of experiments (1917) was with diets derived solely from a mixture of a leaf and a seed ground together so that the two parts of the ration could not be eaten separately. In marked contrast to the failure of animals to grow on any mixtures of seeds, it was found that a mixture of a seed and a leaf formed a diet on which considerable growth could be secured. Polished rice, which requires supplementing with respect to mineral salts, vitamins and protein before it becomes complete for the rat, was found to induce growth when fed with leaves in the proportion of 60 per cent. of seed to 40 per cent. of leaf. On this simple and monotonous mixture rats grew from weaning time to 83 per cent. of the adult size. One female produced two litters, but allowed them to die. A mixture of rolled oats 60 per cent. and alfalfa leaves 40 per cent. makes a better ration. On this mixture rats grew to the adult size and reproduced and reared young. One female reared 14 out of 17 in 3 litters. Maize and alfalfa leaf or wheat and alfalfa leaf were not so satisfactory for growth as the mixture containing oats. Mixture of the alfalfa leaf with peas or navy beans gave poorer results. The peas and beans were cooked and subsequently dried before feeding.

A point of interest is that the bones of these vegetarian rats were of very good quality. There was no evidence of rickets in the young. This disease develops in young animals restricted to a diet of cereal grains. The nature of the defects in the diet which cause this disease will be considered later. It is sufficient to say that a proper adjustment of the mineral elements in the foods, and the presence of an organic factor which exerts an important influence on bone formation, are indispensable conditions for normal skeletal development. The results of this experiment

demonstrate that the mineral content of the leaf component of the vegetarian diet supplemented the inorganic nutrients of the seed portion, so as to favor normal bone development. It is also evident that this diet contained sufficient of the organic factor concerned with the metabolism of bone. In China and other parts of the Orient where green, leafy vegetables constitute a very important part of the food supply, rickets is almost unknown in children, whereas it is common in Europe and America, where the tendency is to derive a large part of the food supply from milled cereals, meats, legume seeds, and tubers.

FIG. 7.—This photograph shows the degree of success which has been attained in securing growth, reproduction and rearing of young in an omnivorous animal, the rat, with a diet of strictly vegetable origin. The diet is described on page 151. The data available seems to demonstrate that successful diets of plant origin can be secured only when the leaf of the plant is a prominent constituent of the diet. Those parts of the plant whose functions are those of storage organs (seeds, tubers and roots), do not serve as adequate diets, even when there is a wide variety in the food supply. The leafy structures are so constituted as to correct the deficiencies of mixtures of seeds, tubers and roots.

The albino and her daughter are shown above, and her granddaughter below. Young in the fourth generation were successfully reared, with no apparent diminution in vitality.

These experiences made it evident that there is nothing in vegetarianism per se which makes it impossible for an animal of the omnivorous type to be satisfactorily nourished. It is only necessary to make the proper selection of food-stuffs, and combine them in the right proportions. In the experiments in which the diet was made up of so simple a mixture as a leaf and a seed, optimum growth, reproduction and rearing of young were in no case secured. McCollum and Simmonds (unpublished data) have the results of an extensive series of feeding trials in which the rations were made up of one seed, one leaf and one

legume (pea, bean) in various proportions; but they have failed to reveal any mixture which is quite the equal of that used in the first experiment, viz., maize 50 per cent., alfalfa leaf 30 per cent., and peas 20 per cent. It is possible that better mixtures of vegetable foods may be found, but these results show definitely that for the omnivorous type of animal, whose digestive tract is small, the consumption of sufficient volumes of leafy foods is impossible. It is by no means a simple matter to derive the diet entirely from the vegetable foods and secure optimum well-being. These experiments also demonstrate that wide variety may be of little value as a safeguard in nutrition.

Cause of Failure of Slonaker's Rats.—There are several reasons why Slonaker's rats did not thrive on their vegetarian diet. It was low in protein. The leaves, which formed the only components of the food containing enough mineral elements to support growth, were fed in the fresh condition. In this form the water content and bulk are so great that it would be impossible for an animal whose digestive apparatus is no more capacious than that of a rat to eat a sufficient amount of leaf to correct the inorganic deficiencies of the rest of the mixture. Physical limitations likewise led to failure to secure enough vitamin A. This was not so important a fault as the inorganic deficiencies but was a contributing factor. Success would also turn upon the ability of the animals to instinctively select the best proportions of the food-stuffs offered them. Appetite is by no means a safe guide to the selection of food.

Evvard's Studies on Appetite as a Guide to the Selection of Food.—Evvard studied the extent to which appetite and instincts of the hog aid in the selection of food (1915). As a rule in all these trials the animals were given a choice of only three foods: a cereal grain, a protein-rich food, and a plant leaf. The data secured suggest that there is a basis for the belief that selection by the animal itself is worth taking advantage of. The value of the leaf in the food supply of the growing pig could not have been explained prior to the studies of McCollum and his associates with simplified diets and with diets restricted to a single food-stuff. Feeding such diets with single and multiple food additions led to a knowledge of the exact nature of the dietary faults of each.

Carotinemia.—Miura (1917) reported that orange yellow pigmentation of the skin, which has been described by Beatz under the term "aurantiasis cutis" is due to coloring of the

subcutaneous adipose tissue by carotin present in mikan, squash, laver, etc. Due to the affinity of fats for carotinoids, he suggested that the term carotinosis is more nearly exact than the term carotinemia. This excessive yellow pigmentation has since been repeatedly observed, especially among persons who eat too constantly of carrots, squash, and other yellow pigmented vegetables. Hess and Meyers (1919) reported carotinemia in hospital infants receiving large amounts of carrots; when carrots were omitted from the diet the pigmentation disappeared. Hashimoto (1922) says pigmentation of the skin has long been observed among vegetarians in Japan, where Citrous nobilis or unusual amounts of squash are used as a partial substitute for rice among farmers. The condition simulates jaundice but differs from it in that the palms and the soles are more deeply pigmented than the rest of the skin, and the whites of the eyes are not colored as in jaundice. Palmer and Kempster (1919) made a detailed study of the carotinoids in the diet in relation to the color of egg yolks, and the ear lobes, shanks, etc., of birds.

Wu and Wu (1928) have studied the possibility of constructing a purely vegetarian diet from Chinese food-stuffs which would be comparable to a well-balanced mixed diet in promoting growth in rats. These Chinese physiologists were interested in the problem because most Chinese people are largely vegetarian. All the common cereals and legumes, and some 20 varieties of vegetables were fed in the experiments, but no combination of them comparable with their standard diet was found. As far as growth was concerned, these studies indicated a greater degree of success than had been attained hitherto with purely vegetarian experimental diets for rats. They conclude that no vegetarian diet is known at present which would afford optimum nutrition for an omnivorous animal such as the rat. They confirm the observations of McCollum and co-workers that unless the leaf is added in liberal amounts together with a protein-rich legume such as the soy bean, growth is far below normal. These results with an omnivorous creature which was similar in its metabolism to that of man seem to justify Wu and Wu in their conclusion that optimal nutrition of human beings cannot be obtained with purely vegetarian diets.

Vegetarianism Viewed from Wrong Angle.—It will be clear from the discussion in this chapter that the vegetarian diet has been considered from the wrong viewpoint. It is possible to

make a fairly satisfactory diet of foods derived entirely from vegetable sources, but it is not easy to do so. It would be difficult for man to eat enough leafy foods to enable him to succeed with a strictly vegetarian diet. Such a diet will generally be low in protein. When one adheres to such a regimen, the addition of small amounts of meat will be valuable; and eggs, because of their relative richness in vitamins A and B, will often be a more effective supplement. The same may be said of small amounts of glandular organs.

Many of the people of China and of Japan, because of poverty and overcrowding, can afford only small additions of fish, poultry, eggs, or the flesh of mammals to a diet which consists in great measure of vegetable foods. From an early age the children are schooled to eat large amounts of leafy vegetables. Many weeds are eagerly sought after and trees are stripped of their buds in spring to supplement the supply of spinach, cabbage, and other leafy foods. An alfalfa field seeded by the Rev. Mr. Holding (personal communication) in north central China was eagerly appropriated for human food, although it had been intended to serve as a forage for animals. Millions of people in Asia have learned the unique nutritive value of green plants, which we in America have never learned to appreciate.

CHAPTER XI

THE DIETARY DEFICIENCY DISEASES—Vitamin A

Xerophthalmia (Keratomalacia)

Investigations on Lipin-free Foods.—Stepp (1909) in the laboratory of Hofmeister carried out the first experiment which demonstrated the indispensability of some substance contained in lipoid extracts of certain natural foods. He believed the fat-like bodies, the lipins, including lecithin, kephalin, cholesterol, cerebron, etc., to be indispensable dietary components. Most food-stuffs of animal or vegetable origin contain these lipins. Stepp sought to discover what would happen if they were eliminated from an otherwise adequate food mixture.

Lecithin contains combined in its molecule glycerin, fatty acid, phosphoric acid, and an organic base called choline. Cephalin closely resembles lecithin, but yields on hydrolysis a different organic base, amino ethyl alcohol. Cholesterol, chemically an alcohol, has certain physical resemblances to the fats. Cerebrosides are compounds containing in their molecules a fatty acid, a sugar (galactose) and an organic base (sphingosin). All of these are soluble in alcohol, but they differ to some extent in their solubility in the other solvents named. All of these substances are abundant in brain and nerve tissue.

Stepp prepared a bread from milk and wheat flour, and another from milk and "protomol," a protein-rich preparation from rice, and extracted them thoroughly with alcohol and with ether. Mice were fed either the extracted or the unextracted breads; those fed the extracted food died within 30 days, whereas those fed the untreated bread remained healthy. Stepp interpreted these results to mean that certain lipins are essential in the diet. He sought to discover which of the individual lipins was indispensable for the maintenance of health.

The method employed was to restrict mice to the lipin-free diet plus one or more of the individual lipins which can be prepared in a state of purity. He tested lecithin, cholesterol, cephalin and cerebrosides, but was unsuccessful in making the

extracted diet complete by adding one or more of them. He made an extract of egg yolk by shaking with cold alcohol, and divided the solution into two portions: one was heated with 95 per cent. alcohol for 2 days in a water bath; the other was heated only to evaporate the alcohol. Each preparation was added to the extracted bread and the two mixtures were fed to mice: those fed the heated preparation died within 30 days, whereas those receiving the unheated preparation remained healthy. The substances dissolved from the bread by alcohol and ether were changed by prolonged heating, so that they no longer rendered the extracted breads complete for the nutrition of mice; what are now known as vitamins A and B were apparently destroyed.

Birds Produce Fats and Complex Lipins Synthetically.—Fingerling (1912) and independently McCollum, Drescher and Halpin (1912) showed that birds may be satisfactorily nourished on diets very poor in lipins. The latter workers fed three half-grown hens exclusively on a mixture of polished rice and skim milk powder, the latter having been extracted with ether to render it essentially free from fats. On this diet the hens increased their weights about 33 per cent., and laid 57 eggs, which collectively contained 82.95 gm. of lecithin and cephalin. This is several times the amount of these substances contained in the entire bodies of the hens. Both neutral fats and complex lipins must have been produced synthetically by the birds.

Fingerling fed full-grown ducks a diet consisting of 300 gm. of cooked potatoes, 80 gm. of cooked blood albumin, 300 gm. of boiled starch, 10 gm. of calcium phosphate and 10 gm. of calcium carbonate per bird per day. One was estimated to have deposited in her eggs 302.3 gm. of lecithin, and another 246.0 gm. The birds remained healthy during the egg-laying period. There was no doubt that the ducks had synthesized large amounts of complex lipins.

Do Nutritive Requirements of Pigeons Differ from Mammals?—Stepp (1916) inquired whether pigeons differ in their nutritive requirements from mammals. He fed 3 pigeons exclusively on dog biscuit which was extracted with hot alcohol; all were dead of polyneuritis within 43 days. A pigeon on the same food was unable to stand on the thirty-eighth day because of polyneuritis. It was then given doses of a commercial "vitamin" preparation (oryphan) plus an alcoholic extract of egg yolk to furnish the lipins which he believed to be indispensable for

mammals. The bird recovered rapidly and remained in good condition until the ninety-second day of the experiment. It was then fed unextracted dog biscuit until the ninety-eighth day. The diet was then changed to extracted dog biscuit supplemented with oryphan, but without the lipin extract. The pigeon had been on experimental diets for 117 days; the body weight at this time was 271 gm. It still ate well, but its flight was unsteady. Unextracted dog biscuit was fed for 2 days. The pigeon was then given the extracted dog biscuit without the addition of either lipins or "vitamins." The result was a rapid loss in weight, and the development of polyneuritis. Death occurred on the two hundred and thirty-third day.

From these observations Stepp drew the unwarranted conclusion that the bird does not require lipins which are indispensable for mice. The loss of weight of his pigeon shows that its nutrition during this part of the experiment was not satisfactory. These results do not warrant the conclusion that there is a difference in the nutritive requirements of birds and mammals.

In Stepp's investigations the element of growth was not included. He studied the nutritive requirements for maintenance of adult mice and pigeons. His results are, nevertheless, of great importance. Stepp, in his early studies, interpreted the results to indicate that only certain lipins were necessary to supplement successfully a diet of purified food substances. When Funk demonstrated the necessity of a protective vitamin for the prevention of beri-beri, Stepp extended his studies to include "vitamins" in addition to lipins. In November, 1915, he presented data showing the necessity of "vitamins" as well as certain lipins in the diet for maintenance. McCollum and Davis simultaneously demonstrated the necessity for growth or maintenance in rats of a fat-soluble and a water-soluble substance.

Earlier Views of Fat in Diet.—McArthur and Luckett (1915) misjudged the peculiar nutritive value of butter fat as a source of an unidentified dietary essential. They added butter fat to a diet free from substances soluble in ether and alcohol, and observed that mice died as soon as they did on the lipin-free diet. The ration was made adequate by the addition of an alcoholic extract of egg yolk or calf brain. They, like Stepp, drew the conclusion that butter does not contain a substance indispensable for the nutrition of the mouse. The explanation

of their results was finally brought out by the studies of McCollum and Davis (1915). When butter fat or an ether extract of egg yolk was added to a diet of purified food-stuffs, only one of the unidentified dietary essentials (vitamin A) was added. When an alcoholic extract of egg yolk was added, both vitamins were given. Since the addition of either of the factors A and B alone does not make a diet of purified food-stuffs complete, the animals fed one addition died, and there was no evidence to show that the butter fat had any special properties. It was difficult to discover that in the one procedure one essential substance, and in the other, two were being added, and that the presence of the two was the determining factor in the outcome of the experiment.

Osborne and Mendel (1920) made a study of diets poor in fats. Rats were fed ad libitum a dry mixture of starch paste 72, meat residue thoroughly extracted with water 23, and salt mixture 5 per cent. The animals also received daily 0.4 gm. of brewer's yeast, 0.2 gm. of alfalfa leaves, as sources of vitamins A and B, and also 0.2 gm. of cane sugar. The control animals were fed a similar diet, but the meat residue was extracted 5 times with ether containing a little ethyl alcohol. These rats grew as rapidly as on normal diets. The results warranted the conclusion that if true fats are essential for nutrition during growth the minimum necessary must be exceedingly small.

Drummond and Coward (1921) found that young rats could grow from weaning to maturity on diets deprived as far as possible of neutral fats.

It is obvious, from the data presented, that the peculiar value of certain fats for the promotion of growth or of health, does not lie in their fats or complex lipins, but must be due to the presence in them of substances of unknown nature. We recognize three fat-soluble vitamins, A, D and E.

Winters, Smith and Mendel (1927) have reported an interesting study of the measurements of rats stunted by 4 types of deficient diets: (1) low calorie, (2) low protein, (3) low lysine, and (4) low salt. The growth of the body systems and organs was affected differently by different inadequate diets. There was persistent skeletal growth on all the diets used but this was not at the expense of the organs studied.

Levine and Smith (1927) succeeded in inducing normal growth in rats from 30-180 gm. using diets containing 86 per cent. of the total calories in the form of fat. The animals utilized 98 to

99 per cent. of the ingested fat and showed no histological changes in the liver or other organs.

Xerophthalmia in Man.—When animals are restricted to a diet lacking in vitamin A, there develops after a few weeks a pathological condition of the eyes. This is so characteristic that it must be regarded as one of the manifestations of a specific syndrome. Livingstone (1857) in describing the hardships en-

Fig. 8.—Illustrates a baby observed and treated by Dr. C. E. Bloch, of Copenhagen. The child was suffering from severe xerophthalmia due to being confined too largely to a diet of separator skim milk supplemented with cereals. The right eye is filled with leucocytes and presents a yellow appearance. The left is swollen shut. The right eye was affected with a corneal ulcer. The sight of the right eye was lost.

dured in his explorations in Africa, relates that his party, while suffering from exposure to the weather, lived for a time on sugarless coffee, manioc roots and meal, and states, "the eyes became affected (as in the case of animals fed on experiment on pure gluten or starch)." We have been unable to discover a description of the original experiments with which Livingstone was familiar. It is remarkable that at this early date he was familiar with the xerophthalmia which results from faulty diets. Spicer (1892) states that after measles or whooping cough with bronchitis when there had been serious exhaustion, the cornea not infrequently became damaged by large perforating ulcers.

He states that after serious malnutrition the cornea might slough spontaneously—a condition not uncommonly seen among nurslings in Russia where the mothers practiced long religious fasts. In England it was rare except among the hand-reared children who had an insufficient nitrogenous diet. The affection generally attacked both eyes of children from 4 to 9 months old;

Fig. 9.—Illustrates a baby from the practice of Dr. Bloch, which had suffered from an attack of ophthalmia of dietary origin, and was cured by administration of fat-soluble A as butter and cod-liver oil. The disease had, however, progressed so far that the sight of the left eye was destroyed. The right eye was somewhat damaged.

it began with a dryness of the conjunctiva with patches of froth on its surface and with night blindness. Soon the whole cornea became opaque and perforated. The termination was often fatal. In the treatment which he applied the principal feature was to increase the nitrogenous portion of the diet with meat juice or raw chopped meat in addition to supplying meat and cod liver oil.

Mori (1904) described fourteen hundred cases of xerophthalmia

among children in Japan between the ages of 2 and 5 years. They exhibited keratomalacia as well as xerosis of the conjunctiva. This syndrome is known in Japan as hikan. Mori states that the disease does not occur among fisher folk, but among people whose diet is largely of vegetable origin. The children suffered from diarrhea in the summer time in addition to the eye lesion. The diet consisted of "rice, barley, cereals, beans and other vegetables." Prompt relief of the eye symptoms was observed when cod liver oil was administered; chicken livers and eel fat were also found to be effective. The successful treatment of xerophthalmia in these children by the administration of cod liver oil or by the feeding of a glandular organ, such as liver, affords very strong evidence that the ophthalmia was the same condition as that produced experimentally in animals.

Experimental Xerophthalmia in Animals.—Falta and Noeggerath (1906), before the vitamin hypothesis was formulated, described a conjunctivitis with crust formation beginning about the fourth week and lasting until death in rats fed a diet which, from their description, must have been very deficient in vitamin A. Knapp (1909) restricted rats to a diet of purified food substances and described a severe conjunctivitis which developed as the nutrition of his animals failed. In other experiments lecithin, cholesterol and nucleic acid were added to the basal diet, and although the animals suffered from malnutrition and died, the characteristic eye symptoms did not appear. This is probably to be explained by the presence of vitamin A as an impurity in the lipins which he employed.

Freise, Goldschmidt and Frank (1913) observed the same eye disorder in experiments with rats which they restricted to a diet similar to that used by Knapp. They stated that in about 3 weeks from the beginning of the experiment the eyelashes of the animals began to fall out. There was retraction of the eyeballs from spasm of the extrinsic muscles. In 5 to 6 weeks the sclerotic coat became dry, the cornea clouded and finally ulcerated, but without marked inflammation. The fur became rough and the animals succumbed to malnutrition. Histological examination of the sclerotic coat revealed keratomalacia as it is seen in man. In cases where the ulceration of the sclera of one eye was well developed, and was beginning to show in the other, the process could be entirely arrested by the administration, suggested by the work of Hopkins, of 2 c.c. of skim milk per day. These workers believed that the condition developed only in

young growing animals, and that it was closely related to the phenomena of growth. They attributed the condition to lack of an "accessory" substance in the diet.

Osborne and Mendel (1913) called attention to the prevalence of inflammation of the eyes of rats restricted to a diet of purified proteins, "protein-free milk," lard, and starch. They referred to this as a "type of nutritive deficiency exemplified in the form of an infectious eye disease prevalent in animals inappropriately fed," and stated that the condition was speedily alleviated by the addition of butter-fat to the diet.

Xerophthalmia in Danish Children.—Bloch (1917) described 50 cases of xerophthalmia in children in the vicinity of Copenhagen during the years 1912-16. Most cases were infants under one year of age. They were suffering from severe malnutrition. The skin was dry, shriveled and scaly. Their diet consisted of separator skim milk, practically free from fat. The milk had been pasteurized and heated again in the home before it was fed. Oatmeal gruel and barley soup constituted the other important ingredients of the diet. These infants were doubtless suffering from lack of several dietary factors. Such a diet would contain relatively little vitamin A, the amount depending on the nature of the feed of the cows, the thoroughness of skimming, the amount of skim milk which was consumed, and the extent to which the heat treatment destroyed it. If the consumption of cereal was large, the amount of milk taken would be too small to insure a satisfactory inorganic content in the diet. The diet which Bloch described would be practically free from vitamins C and D. The infants were probably in a borderline condition of scurvy, rickets, and beri-beri, along with very pronounced ophthalmia. *In the past we have failed to appreciate the seriousness of the condition of malnutrition which falls but little short of a deficiency disease.* The effects on vitality may be very pronounced in cases where a faulty diet is adhered to until clinically observable symptoms are about ready to appear, although no clear cut lesions are detectable.

Bloch believed the malnutrition in these infants to be due to lack of fat in the diet, since the eye trouble could be relieved by the administration of cod liver oil, whole milk or cream mixtures. In the light of our newer knowledge there is no reasonable doubt that vegetable fats such as olive oil, cotton-seed oil, soy bean oil, coconut oil, etc., would have been of no therapeutic value. The prominence of skim milk suggested to Bloch the paucity of fat

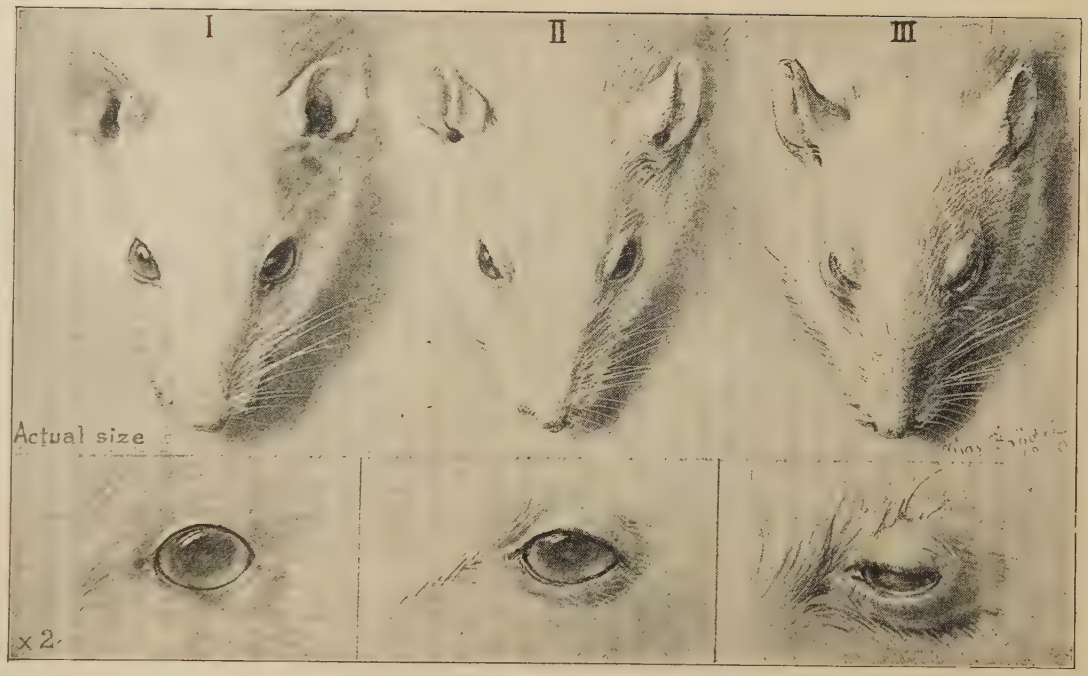


FIG. 10.– Illustrates the appearance of the exterior of the eyes as ophthalmia of dietary origin develops. On the left is shown the normal eye of the rat. In the center is shown the puffiness around the eyes which denotes that ophthalmia is about to appear. On the right the extreme edema of the eyelids is shown. When the latter condition is reached the cornea usually ulcerates and a bloody exudate seals the lids shut. The eyes become filled with leucocytes. The introduction of a fat containing vitamin A leads, in the earlier stages, to prompt recovery from this condition.

in the diet; he fortunately chose two effective fats, cod liver oil and butter fat, for administration to his infants. Bloch (1919) divided 32 healthy children of ages 1 to 4 years in an institution in Copenhagen into two groups for nursing and feeding: in group 1 during May and June, 8 cases of xerosis developed, while those in group 2 remained free from the disease. The diets were similar except the breakfasts: those in group 1 were given oatmeal porridge and rusks, whereas those in group 2 were given beer soup and some whole milk. Apart from the whole milk given to group 2, the only fat supplied was margarine from vegetable fats. Thus, unwittingly, a human experiment was carried out *en masse* corresponding exactly to feeding experiments on animals. The fat in the whole milk in group 2 protected the children against xerophthalmia. The ophthalmia in group 1 was cured with cod liver oil. Bloch (1924) reports a clinical experience with xerosis in Danish children and gives an analysis of 100 cases observed between 1909 and 1920.

Vitamin A Starvation and Xerophthalmia in Rats.—McCollum and Simmonds had repeatedly observed the development of this peculiar form of ophthalmia in animals subjected to faulty diets. They collected numerous observations, all of which pointed to the belief that the eye disease in question is non-infectious, and that it could more satisfactorily be explained on the basis of specific starvation for vitamin A than on any other. Many groups of rats in various degrees of enfeeblement or arrested development resulting from restriction to faulty diets of various kinds, failed to develop the disease, when others in adjacent cages were suffering from it. It occurred only in those animals whose diets were deficient in vitamin A. They (1917) correlated these observations on rats with those reported by Bloch and by Mori, and concluded that the xerophthalmia or keratomalacia produced experimentally in animals is the analogue of the condition reported in man. On this evidence they formulated the view that this type of xerophthalmia is a manifestation of a deficiency disease due specifically to lack of vitamin A.

Requirements of Adult Rats for Vitamin A.—Drummond (1919) stated that adult rats need little if any vitamin A for the maintenance of health. Stepp's observations indicate the reverse. McCollum and Simmonds (1917) found, when the sole source of A and protein was 33 per cent. of millet seed in the food that adult rats developed severe xerophthalmia after 60 days, although millet seed contains demonstrable amounts of A.

FIG. 11.—The rations of these two rats from weaning time were exactly alike except in the character of the fat which they contained. The one on the left was given 5 per cent of sunflower seed oil. The one on the right was given 1.5 per cent of butter fat. Butter fat, egg yolk fats and the leaves of plants contain a dietary essential, the chemical nature of which is still unknown, which is necessary for growth or the maintenance of health. This substance is known as vitamin A, and is not found in any fats or oils of vegetable origin. A lack of this substance in the diet causes the development of a peculiar eye disease known as xerophthalmia. The animals were the same age when photographed.

It is possible that adults require less A for maintenance than young animals do for growth, but this is not proven. An animal on a diet satisfactory except as to its content of A will not show signs of a lack of it for a longer time than if a second factor in the diet were below the optimum in quality and quantity. The extent to which the second factor deviates from the optimum determines whether it would promptly or slowly show the effects of faulty nutrition. If at the same time there should exist a deficiency in a third factor, the effects of lack of vitamin A would become increasingly apparent. In the interpretation of quality in the diet it is essential to realize that there is an interrelation of factors. In short experiments it is easily possible to overlook deficiencies which are of small magnitude, which, nevertheless, manifest themselves later in the life history of the individual.

Vitamin A and Nitrogen Metabolism.—Morgan and Osburn (1925) studied the effect of vitamin A deficiency upon the character of nitrogen metabolism. Their data suggest that in the absence of vitamin A the animal organism fails to produce purine-containing compounds from the ordinary sources, perhaps arginine and histidine, but continues to utilize over again such portions of discarded purine ring-containing substances as are ordinarily excreted in the form of allantoin. The portions of these compounds which are oxidized only to uric acid are apparently no longer usable and continue to be excreted in proportion to the amount of destruction of cellular material in vitamin A-deficient as in normal animals.

Animals Store Vitamin A.—Sherman and Kramer (1922) state that young rats at weaning time have a store of vitamin A which promotes growth for a period when they are deprived of it. The survival period of full grown rats deprived of vitamin A depends upon the amount available to them before the beginning of the test. Goldblatt and Soames (1923) found it stored in the liver in proportion to its content in the diet. Half a gram of liver from rats which were fed a vitamin A-free diet and which had ceased gaining weight for 3 weeks contained no vitamin A. Half a gram of liver from rats which were fed a vitamin A-free diet, but which were irradiated with the mercury lamp for 4 weeks after they had ceased growing, did contain an appreciable amount of the vitamin; but in spite of this result, it was concluded that other factors were operating, and that vitamin A was not synthesized by irradiation. Steenbock, Sell and Nelson

(1923) examined rat livers from animals on a diet poor in vitamin A and on one containing it in abundance. The young of rats fed 5 per cent. of cod liver oil in the stock rations grew almost normally for about 4 months when placed upon a diet very low in vitamin A, whereas young from mothers on the same stock ration without the cod liver oil did not grow at all.

Vitamin A Requirements of Infants.—Hess and Unger (1920) hold the view that in human nutrition there is not likely to be a deficiency of vitamin A sufficiently pronounced to be of importance except under exceptional circumstances—as in times of war. They base their conclusion on an experiment conducted with 5 infants who were fed dried skimmed milk (krystalac) 150 gm.; sucrose 30 gm.; cotton-seed oil 30 c.c.; autolyzed yeast 15-30 c.c.; orange juice and cereal. The autolyzed yeast provided the anti-neuritic B, the orange juice supplied C, and the olive oil furnished fat. The diet was assumed to be almost free from vitamin A, but adequate otherwise. The babies were from 4 to 9 months old. They were confined to this diet for periods varying from 5 to 9 months. They grew in a fairly normal manner. Rickets was not observed in any of them. From this experiment Hess and Unger were led to doubt whether more than traces of vitamin A are necessary in the nutrition of the human infant when other factors in the diet are satisfactory. The assumption that the diet employed was nearly devoid of vitamin A is erroneous. Ross (1921) described 4 cases of nutritional keratomalacia in infants and gives a review of the literature pertaining to the disease. The children observed by him had been fed almost exclusively on condensed milk. Schwartz (1925) also reports an interesting case of nutritional keratomalacia.

Eye Affections in English Schools Referable to Faulty Diet.—Cramer (1924) describes epidemics of eye affections which occurred in England during a period of 40 years in various industrial schools. The last recorded outbreak was in 1911 and was investigated by McNeil and McGowan (1913). The condition of the children was diagnosed as "distorted pneumonia" which varied from a fulminating rapidly fatal type to an abortive or latent type. Special mention was made of the prevalence of a chronic granular conjunctivitis. They recognized the noncontagious nature of the epidemic but were unable to explain the nature of the disease or recommend rational treatment.

Butter Consumption and Xerophthalmia in Denmark.—Widmark (1924) states that the consumption of milk fat (but-

ter) in Denmark before the World War and to the end of 1916 steadily declined and reached very low levels in the last year. At the same time the number of cases of xerophthalmia reached their maximum. Then came a complete change: during 1917 and 1918 and part of 1919, the exportation of butter was forbidden, and more milk fat was consumed in the country than in the years before the war. Xerophthalmia disappeared almost completely but reappeared in 1920 when butter was again exported.

Vitamin A and Night Blindness.—A widespread condition which seems to be related to a deficiency of vitamin A is hemeralopia, or inability to see by subdued light. This is common in several parts of the world, and has been frequently referred to faulty nutrition. Thus, Krienes (1896) attributed the disease to malnutrition. DeGouvea (1883) gave a résumé of the literature of the subject, and described many cases of hemeralopia among negro slaves working the coffee plantations of São Paulo, Brazil. They were restricted to a diet of beans, pork fat and maize meal. Slaves on other plantations who were better fed did not suffer from the disease. Little (1912) mentioned the prevalence of night blindness (nyctalopia, often inaccurately called hemeralopia) among the people of Newfoundland; he also stated that the diet was sufficiently poor to make beri-beri and scurvy of common occurrence. We are informed by Dr. Grenfell that night blindness is common in Labrador. Dr. Appleton (personal communication) also informs us that she has seen many cases of this eye disease in Newfoundland and Labrador. The diet of the inhabitants of these regions consists almost exclusively of canned meats, fish, molasses, tea, small amounts of raisins, and bread made from bolted wheat flour.

The diet in these regions is of a character which one would expect to be dangerously low in vitamin A as well as deficient in other respects. It is essentially a diet derived from the endosperm of wheat, muscle tissue of beef, pork and fish, and molasses. The last article of food, after its drastic treatment in the manufacture of sugar, would not be expected to have any appreciable value as a source of any vitamin. Such a diet is actually so poor in vitamins B and C that beri-beri and scurvy are of frequent occurrence.

Dr. Ann Young (personal communication) has informed us that night blindness is common in the vicinity of Calcutta. The popular remedy consists in poulticing the eyes with juice of

fresh goat's liver and in including liver in the diet. The value of liver for the cure of night blindness has been appreciated in Japan from very early times. The glandular organs of animals are good sources of vitamin A. It is not strange that night blindness in certain parts of the world is due to specific starvation for vitamin A. In Japan, where there is no dairy industry, the supply of A depends largely upon the extent to which leafy vegetables and fish are consumed. Certain edible roots have been shown to contain moderate amounts of this substance, but the amounts of such succulent foods as would ordinarily be eaten by man would scarcely suffice to meet the requirements of the body. The encouragement of the dairy industry in these countries, or the importation of liberal amounts of powdered, dried, or canned milk, and their wider use in the diet, together with the utilization of fish livers and other glandular organs of animals, and of eggs, as adjuvants to the diet, would go far toward the elimination of night blindness, which, provisionally, may be regarded as a specific syndrome of dietary origin.

From early times records have come down to us showing that epidemics of dropsy have occurred during famines caused by drought or war. In the descriptions of this disorder, it has been noted that hemeralopia frequently preceded the development of the edema. It is said that night blindness is endemic in Russia during the long Lenten Fasts. Corneal ulcers and xerosis of the conjunctiva have sometimes been noted among those who suffered from edema due to faulty nutrition, and ophthalmologists have repeatedly referred these eye changes to poor nourishment. These eye troubles in man result from conditions so complicated that it is impossible without further evidence to attribute all of them to specific starvation for vitamin A. There is, nevertheless, a strong presumption that xerophthalmia or keratomalacia and night blindness (hemeralopia, nyctalopia) not infrequently occur as manifestations of a deficiency of this dietary factor.

This conclusion is supported by the frequent occurrence in the literature of ophthalmology of expressions of the belief that malnutrition may cause hemeralopia. Illustrations are found in the association of eye diseases with pellagra by Rampoldi (1885), in the view of Ssaweljew (1892) that night blindness results from fat hunger, in the report of Roussanow (1885) that cod liver oil is an unfailing remedy for hemeralopia, and in the description of "spring-time catarrh" and its associated conjunctivitis by Vetsch (1878). These observations suggest that a diet derived

too largely from cereal products, tubers and other foods having similar deficiencies, induces lowering of vitality, which becomes manifested, among other ways, in faulty vision. Probably not all cases of night blindness are the result of deficiency of vitamin A, but Ackroyd (1928), Hift (1920) and Zak (1920) have reported cases in which this condition cleared up immediately when an abundance of vitamin A was given the patients.

Glandular Adipose Tissue.—Cramer (1920) reported a study of the relation of fasting for vitamin A to changes in the histological structure in certain adipose tissue, which he designates as "glandular." Several histologists have recognized that adipose tissue in certain parts of the body, especially the subpleural tissue, the neck and interscapular region, the axillæ and around the kidneys, presents a pink and lobulated appearance resembling a fresh pancreas, differs markedly from ordinary adipose tissue and can be recognized macroscopically. This tissue is histogenetically distinct from ordinary adipose tissue. It has been described under a variety of names, such as "primitive fat organ," "fat gland," "hibernating gland," "brown fat" and "interscapular fat." This fat has in the embryo a characteristic gland-like structure. Certain species, such as the rat, domestic mouse and the hibernating animals, retain this structure throughout life. In most species, according to Cramer, soon after birth the tissue acquires the appearance of ordinary adipose tissue. This "glandular" adipose tissue is very vascular and is distinct from the ordinary form in its function. Many of its cells are polygonal, present a reticulated appearance, and contain numerous small globules of lipins surrounding a nucleus, in contrast to ordinary fat cells which are oval, and generally contain one large fat globule engorging the cell and pushing the nucleus and protoplasm to one side. The "glandular" adipose tissue appears to contain a larger proportion of anisotropic or doubly refracting lipins than ordinary fatty tissue; the cells of the former are much richer in protoplasm than the common type of adipose cells.

During fasting the "glandular" type tends to persist and shows no obvious diminution, whereas the ordinary type of adipose tissue is rapidly and greatly diminished. In cretins there are circumscribed swellings of the "glandular" adipose tissue in the neck and axillæ, and there appears to be a hypertrophy of this tissue when the thyroid gland atrophies, suggesting a functional relationship between them. Cramer points out also that there is evidence of such a relation between the "glandular" adipose

tissue and the adrenals. There appears to be a close relationship between the lipins of this tissue and those of the adrenal cortex.

When rats are fed a diet of purified food substances, free from all vitamins, this "glandular" tissue loses all its fat and lipins and presents the appearance of a glandular organ. Under the same conditions there is a great reduction of the lipins of the cortex of the adrenals, but these never become entirely free from them unless death occurs. Under the influence of such a nutritive disturbance the ordinary adipose tissues lose all their fat and other lipins and revert to the connective tissue type of cells. Cramer suggests that the "glandular" adipose tissue is a reservoir of vitamin A, since the disappearance of the characteristic lipins from it and the adrenal cortex is followed by death.

Utilization of Vitamin A Introduced Subcutaneously.—Blegvad (1923) reported a most interesting case of xerophthalmia in a man aged 54 who was suffering from cancer of the liver and jaundice; the patient's diet had not been deficient in vitamin A, but xerosis of the conjunctivæ with severe hemeralopia had developed. He was excreting undigested fat and showed other signs of faulty assimilation and was declining in weight. For 6 weeks the patient was given a diet rich in vitamin A without improvement of the eyes. He was then given every other day a subcutaneous injection of 1 c.c. of a preparation believed to be about 100 times as potent as cod liver oil. There was no local inflammatory reaction. After 2 injections the xerosis had disappeared and the hemeralopia improved; after 6 injections the patient could see to walk in the evening and read in dim light. Blegvad affirms that there are cases of keratomalacia in childhood in which it is impossible to avert permanent blindness by treatment with vitamin-rich foods by mouth, and suggests parenteral administration of vitamin A in such cases. Wollmann and Vagliano (1923) found that vitamin A has the same effect when given orally or parenterally except that in the latter case its action is delayed by slow absorption.

Pathological Changes Due to Lack of Vitamin A.—To determine the physiological rôle played by a vitamin, it is necessary to learn what happens to the different tissues in specific starvation for a single factor, no other nutritive principle being involved in bringing about metabolic disaster. Osborne and Mendel (1917) found calculi of calcium phosphate in the urinary tract of 91 animals among 857 autopsies; 43 per cent. of these had not had a satisfactory supply of vitamin A. Mendel (1920)

calls attention to the fact that urinary calculi are exceptionally common among peoples of the tropics, and of the Far East, who live on diets quite unlike the mixed diets common among Europeans and Americans. He points out that these calculi are deposited from neutral or alkaline urines, which frequently owe their reaction to the formation of ammonia as a result of bacterial decomposition. The possibility that the invasion of the urinary tract by micro-organisms results from lowered vitality due to partial or complete starvation for vitamin A is suggested by their findings at necropsy.

McCarrison (1927) states that a diet of whole wheat flour, linseed meal, corn flour, salt and calcium phosphate is very effective in causing calculi in the bladder. This diet is deficient in vitamin A, but rich in its content of calcium and phosphorus. Van Leersum (1927 and 1928) states that rats develop deposits of phosphates and oxalates in the urinary tract very rapidly and almost invariably when fed diets deficient in vitamin A. McCollum, Simmons and Becker (1924-1925) observed calculi in many rats fed 3-5 per cent. of butter fat, or 1, 3, 4, and 10 per cent. of cod liver oil. The diets which produced stones were invariably excessive in their calcium content. The subject is in need of more detailed study.

Stephenson and Clark (1920) made bacteriological and histological studies of keratomalacia in rats. Organisms of various kinds were found. Pus from the diseased eyes in many cases contained a pneumococcus. They believe that when the rat has been on a deficient diet for some time resistance of the cornea is lowered, and pathogenic bacteria which by chance are in the conjunctival sac invade the corneal epithelium and destroy the tissue. Wason (1921) studied the pathology of ophthalmia of dietary origin, but was unable to secure any fundamental data concerning the etiology of this condition. She observed in the eyes of rats selectively fasted for vitamin A, hyalinization or necrosis of the outer layer of corneal epithelium, exudation of serum and cells into epithelium and stroma, and a proliferation of blood vessels and fibroblasts. In advanced cases invariably the anterior and occasionally the posterior chamber were invaded. She states, "the type and virulence of the organisms of secondary infection determine, in part at least, the course of the disease."

Yudkin and Lambert (1922) state that the changes in the eyes resulting from lack of vitamin A do not begin in the cornea but have their origin in the lid. In this respect the sequence of

events is the same as that of some of the more severe types of acute and chronic conjunctivitis which are frequently complicated by corneal injury with infection and ulceration. They also say that the lacrymal gland may be the seat of either degenerative or inflammatory pathological changes. There was variation in size, form, and staining properties of the cells, which they believe referable to functional disturbances related to the ophthalmia. Yudkin (1922) describes incipient changes in the eyes of rats deprived of vitamin A as similar to those seen in xerosis of the conjunctiva and cornea in man, and says that the more advanced picture is that of keratomalacia. Holm (1922) also agrees that the xerophthalmia in rats and infants are identical. Ghose (1922) says that although beri-beri, scurvy and rickets are all encountered in Bengal, they are not widespread. He emphasizes the low resistance of Bengalese to various infectious diseases and states that the presence of certain forms of eye diseases suggests that the industrial classes may be living at or below the danger level of vitamin deficiency.

Mori (1922-23) studied histologically the eyes of rats in various stages of specific starvation for vitamin A. He found the first observable change to be a tendency for the lacrymal gland to go into a resting state, and to cease to produce tears. Subsequent changes in the eyes can be accounted for as the result of loss of function of the tear glands. When the secretion fails, the conjunctival sac is no longer washed continuously as under normal conditions, and bacteria quickly grow there in great numbers. This growth stimulates a migration of leucocytes which accumulate in the eye chamber, causing the well-known hypophyon, which is visible as a yellowness in the pupil. Some of the leucocytes migrate through the outer coating of the eyeball and into the conjunctival sac, there to undergo dissolution with subsequent accumulation of albuminous material which tends to paste the eyelids together. The drying of this exudate makes it difficult for the animals to open their eyelids. It was found possible to cultivate ever-increasing numbers of bacteria from swabs taken from the surface of the eyeball day by day during the progressive fasting for vitamin A.

Due to dryness of the eye, there is cornification of the external coat, the cells become flattened and pile up in pseudo stratified form, resembling horny layers of skin. Ulcers regularly form on the cornea during the latter stages, owing to death of the tissue. The ulcers finally perforate and the lens pops out. It

is possible to induce spectacular recovery in animals whose eyes are in a very damaged condition by administering fats containing an abundance of vitamin A. Mori regarded the ophthalmia of dietary origin as the analogue, in certain respects at least, of a condition known as hikan, a human disease common in many parts of the Orient.

Sections of the lids of the eyes of animals suffering from xerophthalmia very often show cystic dilation of the ducts of the Meibomian glands. These cysts are filled with fat—the secretion of the gland cells. The epithelium of the margin of the lid shows evidence of a very marked xerotic process, and the same change is found in the epithelium of the duct. It is very likely that this xerosis may play a part in the occlusion of the duct and the formation of the retention cysts.

The lumina of the acini of the Harderian gland may be either very much dilated and empty, or narrowed by swelling of the secreting cells. Frozen sections of this gland show a remarkable diminution of the fat content of the cells and the lumen. The connective tissue about the gland acini is very often densely infiltrated with round cells.

The mucous cells of the conjunctiva are entirely destroyed in the course of the xerotic process. From these findings it is certain that the entire secretory apparatus of the eyes of these rats is in a state of dysfunction. The secretion from these glands is either very much diminished or lacking. The changes in the lacrymal gland are the most important in the pathological picture—in fact, the changes in this gland would seem to be the cause of the lesions in the cornea and conjunctiva. The failure of the lacrymal secretion explains the increase in number of the organisms found in the conjunctival sac.

Clinically, and at autopsy, the mucous membrane of the mouths of rats suffering from xerophthalmia was dry. In the early stage of the disease the animals sneeze and cough violently; but later the cough subsides spontaneously and after a period of dysponea death ensues. Nevertheless, histological sections of the mucosa showed no more than the normal cornification of the epithelium. On the other hand, the mucous membrane of the larynx and trachea showed xerotic changes.

Mori also examined the salivary glands of rats suffering from lack of vitamin A, since both in nerve supply and in structure these glands resemble closely the lacrymal gland. The submaxillary and parotid glands of the rat are serous; the sublingual

is a mucous gland. In many rats with xerophthalmia either all or some of these glands are either secreting very little or not at all. The secreting cells become much shrunken, the acini are very small and show no traces of secretion. The epithelium of the intra-lobular ducts is shrunken and the cells are irregular in size.

The epithelium of the principal ducts of these glands often shows cornification and desquamation of the thickened superficial cells, so that the lumen of the duct becomes narrow and is often occluded. Dilation of the small ducts occurs frequently in the parotid gland. Coincident with the xerotic changes and with those of the parenchymal cells, the ducts are invaded by bacteria, and small abscesses are formed in the gland.

The other secretory organs, such as the liver, pancreas, bowel, kidney and thyroid, showed no remarkable change except in one case—in one animal the cytoplasm of a small number of cells of the pancreas was very much vacuolated. The reproductive glands of the rat on a vitamin A deficient diet do not function.

Ophthalmia Due to Unbalanced Mineral Content.—McCollum, Simmonds and Becker (1922) observed that an ophthalmia, indistinguishable in its macroscopic appearance from that produced by deprivation for vitamin A, can be produced in rats receiving an abundance of this vitamin, by including ferrous sulfate in the diet. This salt catalyzed the oxidation of vitamin A (1928). Miller (1923) has called attention to a soreness of the eyes in rats kept on a potassium-free diet containing 1 per cent. of cod liver oil and no other source of vitamin A. Since the cod liver oil was mixed in with the ration, vitamin A was probably destroyed by oxidation.

Vitamin A Deficiency and Fertility in Rats.—Reynolds and Macomber (1921) state that decreasing the content of the diet in vitamin A, in protein or in calcium, markedly decreased the fertility in rats. Evans and Bishop (1922) have described a disturbance of the oestrus in adult female rats when fed a diet deficient in vitamin A. These investigators found that there was a prolongation of the oestrus desquamative change in the vaginal epithelium, the smear consisting chiefly, if not exclusively, of cornified cells which in the normal individual characterize the actual period of oestrus and ovulation only. These rats continue to ovulate and to form corpora lutea, irregularly or at intervals approximating the normal. Eckstein (1923) found quantitative underfeeding, viz., lack of fat, vitamin A,

or phosphate to produce changes in the testes which undergo atrophy, show degenerative changes and fail in spermatogenesis. In the female rat the same conditions did not lead to any essential impairment of function. The diets of Eckstein were also low in vitamin E which complicates his results. Simonnet (1925) concludes that there is no parallelism between changes in weight and in size of rats during deficiency of vitamin A. Growth continues when the weight is stationary or decreasing. The different parts of the skeleton are affected in different degrees. Among the organs, the thymus and testicles are most injured. The variations in the organs during vitamin A deficiency differ from those caused by under-feeding with an adequate diet, but Simonnet was unable to draw any conclusions concerning the mechanism of the action of vitamin A. Old animals are much less affected than young ones, and males are less sensitive than females to vitamin A deficiency; he states that this is not true of castrated females. With regard to the development of the genital organs, males are more sensitive than females, the former becoming sterile under dietary conditions which permit of fertilization of females. Gestation does not proceed normally under such conditions and generally results in the death of the fetuses.

Evans (1928) has continued his studies on the effects of inadequate vitamin A on the sexual physiology of the female rat. He shows that the constant appearance of the cornified cells is due to lack of A and not D. Lack of A, unlike lack of E, injures the reproductive system of the female so that fertilization and implantation often fail. In about one-fifth of the copulations, implantations occurred and the gestations were completed, and normal litters were born. The females apparently had stored some A from the stock diet and had a liberal amount of E present in the diet.

Transference of Vitamin A in Parabiosis.—Dragstedt and Cooper (1923) report interesting experiments involving parabiosis. Two young rats were united by operation so that they had a common abdominal cavity but no union of blood vessels from one to the other. These animals were kept for several months. When one of such a pair was fed an adequate diet, and its mate a diet deficient in vitamin A, the usual symptoms of vitamin A deficiency occurred in the one animal, while its well-fed mate remained normal. They conclude that there is no toxemia in the early stages of vitamin A deficiency, or if so, the toxins do not pass to the control parabiotic twin in sufficient

amounts to induce metabolic disturbances. The vitamin itself cannot pass from one rat to the other.

Blood Pressure in Vitamin Deficiency.—Baldwin, Cook and Nelson (1924) made blood pressure curves from the abdominal aorta in rats suffering from lack of vitamins A and B respectively. Healthy rats maintained a blood pressure of about 84 mm. of Hg. In vitamin A deficient animals there was a slight subnormal pressure, 19 animals giving an average maximum of 75 mm. of Hg. In the B deficient group there was an average maximum of only 34 mm.

Vitamin A Deprivation and Blood-forming Tissues.—Findlay and Mackenzie (1922) state that rats which have survived for a long time on a diet deficient in vitamin A show an almost complete replacement of the hemopoietic tissue by fibrous tissue. There is considerable diminution in leucoblastic cells. If vitamin C is deficient there are patches of gelatinous degeneration and fibrous tissue over-growth throughout the bone marrow, with disappearance of blood-forming cells. Rats fed a diet containing only one-third of the normal amount of protein but otherwise complete show hemorrhagic areas in the bone marrow with disorganization of hemopoietic cells.

Damianovich and co-workers (1923) report that lack of A or B quickly brings on anemia which gradually grows more serious. Vitamin B deficiency produces this effect more rapidly and in a more pronounced degree than deficiency in A. They state that administration of B ameliorates the condition.

Koessler, Maurer and Loughlin (1926) presented data which suggest that blood regeneration cannot take place without vitamin A in the diet. Rapid formation of new blood cells followed the addition of A to the diet of rats long depleted of their vitamin A reserve. They describe a condition similar to pernicious anemia in rats as the specific result of vitamin A deficiency. The anemia appeared to be the result of injury to the secretory glands of digestion, resulting in impaired digestion, over-growth of the alimentary tract by micro-organisms ordinarily found only in the colon, which in turn causes the formation of excessive amounts of toxic products of bacterial decomposition of protein. The capacity of the mucosa to destroy these products is reduced, hence they enter the blood in excessive amounts and injure the hemopoietic tissues.

Vitamins A and B Excreted in Urine.—Cooper (1922-23) found that urine of man or dog on their usual diets contains

little or no vitamin A, but it was present in both when the diets were rich in A. Human saliva from subjects on a diet rich in A contains little or none of it. In dogs the gastric juice from the Pavlov pouch contains vitamin A only when the diet contains it in abundance. Human gastric juice (appetite secretion) contains little A even when the diet includes eggs and milk. The continuous juice contains little or no vitamin. Pancreatic secretion was lacking in A. Muckenfuss (1918) and Van der Walle (1922) found vitamin B in urine.

Vitamin A Deficiency and Intestinal, Aural and Nasal Flora.—Creekmur (1922) found in rats fed a diet deficient in vitamin A a change in the proportion of Gram negative and Gram positive bacteria in the feces. The feces of rats on the deficient diet were dry and hard, which may account for the disappearance of streptococci. There was no change in the proportion of bacteria which fermented glucose, lactose and saccharose. The proportion of H_2S-forming bacteria remained constant. Daniels, Armstrong and Hutton (1923) state that rats fed a diet low in vitamin A may die of bacteriological invasion of ear and nasal cavities before the appearance of xerophthalmia; as the infection advances, it leads to nutritional disaster in which the animal is not restored to a normal condition by feeding rations containing vitamin A.

Other Observations on Effects of Deprivation of Vitamin A.—Hintzelmann (1923) reports that animals fed diets deficient in vitamin A suffer atrophy of the endocrine glands. He observed no hypertrophy of the adrenals and obtained no evidence of failure of kidney function.

Hirai (1923) reports that animals fed a diet deficient in vitamin A for one and one-half months develop marked hyperglucemia. Happ and Wagner (1923) state that in tuberculous children a deficiency of vitamin A has an unfavorable effect upon the disease process, while the abundant administration of this vitamin tends to overcome tuberculosis.

Daniels (1926) reported an interesting observation showing an adverse effect of vitamin A deficiency on absorptive power of the intestine. Artificially-fed babies, like experimental animals on low vitamin A diets, develop infection of the upper respiratory tract. An ether extract of a three weeks fecal excretion of a 7 months artificially-fed baby was prepared. A similar extract of the excreta was made from a 6 months old breast-fed baby who was receiving one teaspoonful of cod liver oil and one

ounce of orange juice daily. These extracts were tested on rats on a low vitamin A diet. The rats fed the extract from the artificially-fed baby recovered, whereas those receiving the extracts from the breast-fed baby died. It appears that the intestine of the artificially-fed baby did not absorb the vitamin A content of the diet so completely as did that of the breast-fed infant.

Deficiency Diseases Rarely Due to Single Dietary Fault.—It is doubtful whether a deficiency disease ever occurs uncomplicated in man or animals. When the list of foods available is greatly restricted and the diet made unsatisfactory to a pronounced degree, the pathological condition produced is a complicated one. The deficiency diseases, with the possible exception of scurvy, can be produced as uncomplicated syndromes, only by carefully planned experimental diets. This is evident from the nature of the deficiencies of polished rice, muscle meats and refined cereal products—the articles prominent in the diet of people who suffer most from beri-beri. Such diets are deficient in mineral elements to a degree that would, in time, lower the vitality; and their content of vitamin A is so low that one obtains a borderline—if not an actual keratomalacia. But in the group of the deficiency diseases a lack of the anti-neurotic substance is the defect which produces the most prompt and striking pathological picture: beri-beri appears, and overshadows all other features of general malnutrition.

In the reported cases of xerophthalmia in man, the inference seems justified that, although many of the infants were doubtless suffering from latent scurvy and underfeeding, lack of sufficient vitamin A was a prominent factor in their nutrition.

In this chapter it has been clearly demonstrated that certain fats contain a substance of unknown nature which is one of the nutrients which the diet must furnish if life is to be maintained. The lack of this substance has caused the appearance of an eye disease among peoples at various times in several parts of the world. It is conclusively shown by experiments on animals that this condition is due to the lack of vitamin A, or to its provision in inadequate amounts in the diet.

CHAPTER XII

STUDIES ON THE PROPERTIES OF VITAMIN A AND THE REQUIREMENTS OF DIFFERENT SPECIES FOR IT

Stability Toward Heat and Chemical Reagents.—Much difference of opinion has existed in regard to the stability of vitamin A toward heat and reagents. McCollum and Davis (1914) observed that hard-boiled egg yolks were still a good source of this vitamin. They also found the melting of butter in a hot water funnel and its filtration during one or two hours, followed by solution in ether, filtration and subsequent evaporation of the ether, did not appreciably lower the content of vitamin A. They reported, therefore, that the substance is fairly stable at high temperatures. They employed in their feeding experiments 3 to 5 per cent. of either butter fat or egg yolk fats as the sole source of vitamin A, and concluded that no extensive destruction could have taken place.

McCollum and Davis (1914) also observed that butter fat may be saponified with alcoholic potassium hydroxide and petroleum ether without the destruction of vitamin A. The soaps resulting from saponification were dissolved in water and shaken with olive oil, samples of which had been shown to be ineffective as a source of A. The olive oil, which had thus been in intimate contact with the soaps of butter fat, was then removed by ether, and the latter evaporated. This olive oil was found to contain vitamin A, since it caused a marked response in growth in rats which had been denied this dietary essential. This experiment and those of Steenbock, Sell and Buell (1921) show that the dietary essential in butter fat is sufficiently stable to withstand saponification in non-aqueous solution.

Osborne and Mendel (1915) passed live steam through butter fat for 2½ hours. Eighteen per cent. of this treated fat supported normal growth.

Vitamin A and Pigmentation.—Steenbock (1919) discovered

that among the vegetable foods the distribution of vitamin A is associated in a remarkable way with yellow pigmentation. Drummond (1919) brought rats into a condition of vitamin A deficiency, and then fed them pure and impure preparations of the pigment carotin. The latter, together with xanthophyll, constitutes the pigments to which the yellow color of many vegetables is due. The animals did not grow, and developed ophthalmia. Carotin is not vitamin A.

Palmer (1914) observed that cottonseed oil, freed from resins, has a golden yellow color and is rich in carotinoids, but is devoid of vitamin A. He also showed that the blood of certain species of animals such as sheep, swine, dog, cat, rabbit, and guinea pig is free from carotinoids. If the vitamin were one of the pigments it could not be absorbed by these species. Dolly and Guthrie (1919) found adipose tissues and nerve cells of these species to be free from carotinoid pigments.

Palmer and Kempster (1919) grew newly-hatched chicks for 6 weeks on a mixture of white maize, white maize meal, white maize bran, skim milk, and bone meal. At this time the birds began to fail, but responded at once when pork liver was added to their diet. The latter was free from carotinoids. At 3 months of age the birds were normal in size. They were then given an occasional feeding of white summer squash and white Spanish onions. There was but a trace of carotinoids in the tissues of the birds when grown, as shown by the lack of pigmentation of the shanks, ear lobes, beaks, etc. At the age of 6 months the hens began to lay, and 17 of them produced 893 eggs in 233 days. One hen laid 88 eggs during this period. The yolks were free from carotinoids. These eggs were incubated. Viable chicks were hatched, which were normal except for the absence of yellow pigment from the shanks, beaks, and other parts. Palmer (1919) criticized the view of Steenbock that food-stuffs containing yellow pigment are necessarily rich in vitamin A. He cites several instances in which this relationship does not hold, and points especially to vegetable oils.

Stephenson (1920) dissolved 300 gm. of butter fat in 1 liter of light petroleum. This was shaken with 4 gm. of fine birch charcoal for 2 hours, filtered and re-treated with charcoal. A butter fat free from color was obtained. The petroleum was distilled off in vacuo and the resulting butter fat, which was as white as lard, was tested for vitamin A. Growth was maintained during 8 weeks, when the experiment was discontinued.

The birch charcoal, therefore, removed the color without removing the vitamin.

Steenbock and Boutwell (1920) had earlier stated that this vitamin is not carotin. In several later papers Steenbock presented data showing the remarkable association of this substance with the yellow pigment of plants. He has shown that when carotin and xanthophyll are separated, vitamin A accompanies carotin, whereas the xanthophyll fraction is poor in it. Steenbock and co-workers (1921) pointed out that carotin of constant melting point through a number of crystallizations was always found to induce growth in rats after growth had been suspended by lack of vitamin A. They point out, however, that the vitamin is not necessarily a pigment, since the A content of butters is not of the same order of magnitude as the pigment content, and that cod liver oil containing little pigment may contain much of the vitamin. They state that A is destroyed neither by hydrogen peroxide nor by reduction with nacent hydrogen. Crystalline acetyl derivatives of constituents of the non-saponifiable vitamin fraction of fats were prepared without destroying the vitamin activity.

Palmer and Kennedy (1921) finally disposed of the question by showing that there is no correlation between pigmentation of certain foods and their content of vitamin A. Approximately normal growth and reproduction were secured in rats on rations free from carotinoid pigments but rich in vitamin A. The liver, blood, spleen, suprarenals, adipose tissue, ovaries and the fat of milk recovered from the stomachs of very young rats were examined with every precaution to determine the presence of carotinoid pigments. Rat milk and the fat separated from the adipose tissue were absolutely colorless. The fat of the blood, suprarenals, spleen and ovaries, and the liver tissue of new-born rats were entirely devoid of color. The livers of adult rats fed a diet containing an abundance of plant carotinoids yielded an oil which was yellowish, but the pigment gave none of the tests for carotin. This substance cannot, therefore, be regarded as in any way related to those plant pigments with which vitamin A may be associated. They instituted growth experiments with rats, using colorless ewe milk fat as the sole source of vitamin A. In other experiments they used, instead of ewe milk fat, colorless egg yolk obtained from hens fed unpigmented food.

The milk of the ewe is almost colorless, but gives a very faint test for plant carotinoids. Nine per cent. of the diet consisted

of this fat, and it was estimated that the pigment content of the ration was only 0.127 parts per million. On this diet rats grew fairly well for 2½ to 3 months, and 3 females each produced litters of 11 young. Two of these mothers were able to nurse their litters, which were reduced to 6. The young grew essentially normally. On a similar diet, in which 15 per cent. of colorless egg yolks served as the sole source of vitamin A, 6 rats grew well, 2 increasing in weight from about 70 to about 230 gm.

Palmer and Kennedy also pointed out certain numerical relations between the carotin content and vitamin A efficiency of various foods. Thus, 15 per cent. of their egg yolks containing no carotin were at least equivalent, as sources of vitamin A, to 85 per cent. of yellow maize, although the latter amount furnished 1,400 parts per million of carotin. Five per cent. of ewe milk fat they estimated to be equivalent, as a source of A, to 1 per cent. of dry spinach. The carotin content of one million parts of rations containing these amounts of materials would amount to 0.1073 and 160 parts per million, respectively. The second of these two diets containing about the same amounts of vitamin A would contain about twenty-two hundred times as much pigment as the former. It appears that further attempts to establish a relationship between a yellow pigment and vitamin A are futile.

Palmer (1922) stated that the yellow lipochrome of cod liver oil is not a carotinoid. The yellow pigment in blood serum of pigeons is xanthophyll. The red pigment in the feet and legs of birds resembles carotin in its solubility but not in other respects. The human organism loses carotinoid pigments by oxidation. Carotinoid skin coloration is due to lowered oxidation processes and superabundance of carotinoids in the diet. Ghose (1922) found vitamin A in buffalo "ghee" which is pure white.

Attempts to Isolate Vitamin A.—Takahashi (1922) described a procedure for saponifying cod liver oil for the preparation of vitamin A. A chloroform solution of his semi-crystalline preparation gives a lipochrome reaction with nitric acid. The substance does not form an acetate and does not absorb bromine but reduces ammonical silver and phosphomolybdate solutions. It is unstable in sunlight and air, taking up oxygen and increasing in weight, with simultaneous loss in reducing power. He believes it to be an aldehyde.

Takashashi and Kawakami (1923) described the preparation of a semi-crystalline product from cod liver oil which they

believe to be nearly pure vitamin A. When 0.08 mg. was administered daily to a mouse dying from lack of vitamin A there was complete recovery in 10 days. The substance contained carbon, hydrogen and oxygen but no nitrogen. It appeared to be of aldehydic nature. It was insoluble in water, but soluble in alcohol, ether, benzene, and acetone. Hattori and Obata (1923) describe several modifications of Takahashi's method for the separation of a concentrated preparation of vitamin A from cod liver oil.

Takahashi (1925), in the laboratory of U. Suzuki, reports the isolation of vitamin A from cod liver oil, butter fat and green laver. He calls the substance biostearin. It has the formula $C_{22}H_{44}O_2$. It is believed to contain two hydroxyl groups, one of which is tertiary, and the other primary or secondary. It is therefore an alcohol closely related to cholesterol. When 0.0001 per cent. of biostearin is present in the diet of the rat, health and growth are maintained. The effect of biostearin he believed to be proportional to its concentration in the diet up to 0.05 per cent., but when this amount is exceeded it has an unfavorable effect. He prepared the benzoate, acetate, hexabromide and ozonide of the substance. Its molecular weight is about 400.

When a small amount of biostearin was placed in a dish and covered with a dry plate, the image of a screen placed between the plate and the biostearin appears on the plate. This was true even when the plate was at a considerable distance from the biostearin. This happened even though the plate was carefully wrapped in black paper. Biostearin sealed in a quartz tube and taken into a dark room gave an image of an object upon a dry plate kept near the tube. The action of the biostearin was less when glass intervened than when quartz was used.

When biostearin was placed in a flask and a current of carbon dioxide was passed over it, and the gas was led through an outlet against a dry plate, no action was observed. When a dilute solution of biostearin was spread upon a dry plate and immediately washed off the plate was not affected. When a dry plate was partly immersed in an alcoholic solution of biostearin the submerged part was not affected but the part of the plate outside the solution was affected. Reduced copper or aluminum foil kept in the same vessel with biostearin was rapidly oxidized. In the environment of biostearin something is formed in the air which gives the KI-starch reaction and the paranitrophenylenediamine reaction. It is believed that it activates oxygen. Biostearin is

a colored substance resembling the carotinoids. An alcoholic solution of the substance gave an absorption band near 3200Å of the ultra violet region. The solution becomes fluorescent when illuminated with light from the iron arc.

Drummond, Channon and Coward (1925) have made a critical study of the procedure employed by Takahashi. They find that vitamin A can be distilled at between 180°-220° C. at 2-3 mm. They identified two alcohols and an unsaturated hydrocarbon in the distillate. They believe the claim of Takahashi that vitamin A is identical with an unsaturated alcohol "biosterin" is unwarranted.

The Chemical Nature of Vitamin A.—Drummond, Rosenheim and Coward (1925) prepared cholesterol from cod liver oil or brain. After 8 recrystallizations these cholesterol samples when irradiated with a quartz mercury vapor lamp induced prompt resumption of growth in rats declining on a vitamin A deficiency diet, when daily doses of 1 mg. per rat were given. Controls fed irradiated material in the same amount made little or no response. The irradiated cholesterol gave certain color reactions not given by cholesterol or oxycholesterol. They found their irradiated cholesterol to be quantitatively precipitated by digitonin, and infer from this that the extent of chemical change effected by irradiation must have been very small. This result would seem to indicate that either cholesterol or some substance which cannot be separated from it by repeated crystallization is capable of activation to form vitamin A by the action of ultra-violet rays.

Rosenheim and Drummond (1925) observed that cod liver oil gives with arsenic trichloride a brilliant ultra-marine-blue color reaction, which they believe to be a specific test for vitamin A. The color persists sufficiently long to permit a colorimetric comparison with a suitable color standard. The cholesterol-free fraction of the unsaponifiable matter from cod liver oil gave the reaction in a dilution of one to two billion. Aeration destroys the chromogenic substance. They state that cholesterol made active by irradiation does not give the above test. In the light of this result it is difficult to understand why they should have been able to confer upon repeatedly recrystallized cholesterol the properties of vitamin A by irradiation as described in the preceding paragraph. They found that dimethyl sulfate, trichloracetic acid, acetyl chloride, and benzyl chloride give blue color reactions with substances containing vitamin A.

Notwithstanding the fact that Drummond was able to arrange a series of oils on the basis of their reaction with arsenic trichloride so as to agree with the arrangement which one would make on the basis of animal tests for vitamin A potency, it seems doubtful whether the test is specific for vitamin A. Antimony trichloride gives a similar blue color with cod liver oil, and has been suggested by Steinle and Kahlenberg (1926) as a reagent for the quantitative estimation of cholesterol colorimetrically, and apparently the color is produced entirely independent of the presence of any fat-soluble vitamin. Hawk (1929) has recently reported a series of observations which cast grave doubt on the value of this color reaction as a criterion of the abundance of vitamin A in an oil. He found that exposure of cod liver oil to oxidation increased the intensity of the blue color formed with antimony trichloride, whereas it is known from the results of animal experiments that oxidation rapidly destroys the vitamin. Hawk is probably correct in his conclusion that the animal assay is still the only accurate method by which cod liver oil or other oil may be assayed for vitamin A.

Further confusion in regard to the chemical nature of vitamin A results from the report of Euler, Euler and Hellstrom (1928) that carefully purified carotin, fed to rats which were declining on a diet complete except for lack of vitamin A, caused a growth response comparable to that resulting from feeding fats containing this vitamin. Even so small a daily dose as 0.005 mg. per rat per day sufficed to induce recovery and fairly rapid growth during 25 days. Carotin, they found, gives the characteristic color reaction with antimony chloride which has been accepted by many as a test for vitamin A. These authors explain the failure of others to secure a growth response by feeding carotin to A-deficient animals on the basis of absence of vitamin D in the experimental rations. This they provided in the form of activated ergosterol. One recalls in this connection the studies of Mendel and Underhill of the effectiveness of crystalline carotin in the cure of a pellagra-like syndrome in dogs.

It is impossible to draw any conclusion at present concerning the merits of the conflicting data purporting to throw light on the chemical nature of vitamin A.

Origin of Vitamin A in Fish Oils.—Hjort (1914) states that in Norway the early spring rise in diatoms leads to the vast multiplication of copepods which form the food of enormous

shoals of small fish which come in to the northern coast (Finmarken) to spawn. These shoals are chiefly composed of capelin and are followed by great numbers of cod, haddock and other species which devour them. The cod's diet also included certain shell fish, larger crustacea, salpa and especially on the Newfoundland banks, squid.

Coward and Drummond (1921) stated that lower plants (marine algæ) containing chlorophyll synthesize vitamin A. Others differently adapted for photosynthesis (red weeds) are not so active in this respect, while those devoid of pigment which play a rôle in carbon assimilation (mushroom) are almost completely deficient. Jameson, Drummond and Coward (1922) reared pure cultures of a common marine diatom (Nitzschia closterium) in sterile sea water, and found this organism able to synthesize large amounts of vitamin A. They draw a parallel between the dependence of land animals on fresh green leaves and that of marine animals on the synthetic activity of the marine flora for their supplies of vitamin A.

Distribution in Marine Products.—Hjort (1922) found vitamin A in sea weed (green algæ, Ulva lactuca and Codium tomentosum), diatom plankton, and the roe of herring and cod. Coward and Drummond (1922) found fish eggs very rich in vitamin A; during the development of the larva fish it is utilized, so that after the content of the yolk sac is absorbed the supply is almost exhausted. They suggest that this is a reason why this stage is so critical a period in the development of young fish. If, in the post-larval stage, or even before the sac is completely absorbed, the fish are given vitamin A, growth and development are satisfactory. Their experiments confirm previous findings that the stores of vitamin A in the tissues of fish can be derived from the food.

In summing up the studies on the origin of vitamin A, Drummond and Zilva (1922) state that it is now generally agreed that the higher land animals derive their supplies of vitamin A from the green plants either directly or indirectly. The probability of a similar relationship existing in marine life has been suggested by Jameson, Drummond and Coward (1922).

Vitamin A in Plant Tissues.—Coward and Drummond (1921) concluded that germination did not appear to increase the vitamin A content of seeds. Etiolated seedlings and leaves deficient in chlorophyll contain little of it. The amount of A in the etiolated shoots of sunflower seeds was not appreciably greater

than in the seed itself. Green shoots apparently synthesize large amounts of vitamin A.

Wilson (1922) demonstrated synthesis of vitamin A in plants. Leaves are rich in it, whereas seeds in general are not good sources of it. Wheat was sprouted in the dark and also in sunlight; after the sprouts were 2 to 3 inches long, they were cut off, dried and fed (5 per cent.) as a source of vitamin A. Either etiolated or green wheat sprouts furnish sufficient A for the growth of rats. This proportion of sprouts represents a quantity of seeds which would be an inadequate source of the vitamin. Vitamin A is produced by the plant with or without accompanying photosynthesis. Such different results with etiolated seedlings suggest that different seedlings vary in their power to produce vitamin A.

Coward (1923) also studied the relation between vitamin A and lipochromes in plants. Instances of the association of vitamin A with lipochromes are: tomato pulp; cucumber skin; the sepals, petals, and petaloid stigmas of the yellow iris flower but not the stamens or pollen; orange juice; red capsicum fruit; yellow capsicum fruit; and the calyx of the ripened fruit of the winter cherry. Cases where absence of vitamin A is associated with absence of lipochromes are: four varieties of mangolds (the flesh of the root); one variety of swede; the flesh of cucumber; inflorescence of the cauliflower; the corollas of ray florets of the Shasta daisy; and the corollas of the ray florets of the purple aster. She states that in the formation of vitamin A some lipochrome is always associated with the vitamin in plant tissues; and where carotin is found, especially in plants exposed to sunlight, the vitamin may be expected to be present. She concluded: (1) light is necessary for the formation of vitamin A in plant tissues; (2) this process can be carried on in the absence of CO_2 and of oxygen in the surrounding atmosphere; (3) the process is independent of the ultra-violet rays of the spectrum (for those wave lengths held back by two plates of window glass) and can be carried on under the influence of the electric light in the absence of sunlight; (5) the presence of chloroform in the atmosphere prevents its formation; and (6) complete absence of calcium salts from the nutrient solution in a water culture of Tradescantia does not prevent the formation of A in the leaves. Widmark (1924) states that when plants lose the ability to form chlorophyll or lipochrome, they also lose their ability to form vitamin A, even if kept in full illumination.

Coward (1927) showed that etiolated shoots of wheat, and white and yellow corn may contain small quantities of vitamin A. Wheat seedlings germinated 2 days and with 3 rootlets and no shoot contain no more A than ungerminated seeds. Ultra-violet rays accelerated formation of A in leaf and plant tissue. Short ultra-violet rays in conjunction with visible rays do not influence the amount of A in plant tissues. Within certain limits excessive illumination of the plant did not have a deleterious effect on vitamin A formation. The A content of etiolated shoots is an inverse function of the temperature at which they are grown. Dye and associates (1927) and several other investigators have confirmed the observation that vitamin A is more abundant in green leaves than in bleached ones. The work of Morgan and Smith (1928) on the development of vitamin A during ripening of tomatoes is of interest in this connection. They found green tomatoes to be very poor in vitamin A, whereas ripe ones contained rather liberal amounts of it. They found that as the lycopin developed in the plucked tomatoes as well as in those ripening on the vines apparently vitamin A developed also. The vine-ripened tomatoes and 4 samples of fruit ripened off the vines under various light conditions appear to be about equally rich in vitamin A.

Effect of Radiant Energy on Growth of Rats and Development of Xerophthalmia.—During the years 1920-1922 there was great interest in the demonstration that ultra-violet rays and sunlight protected both children and animals against rickets. Since rickets had been produced in animals on diets which induced both ophthalmia and rickets, the question arose, "Can ophthalmia be prevented by exposing the animals to active radiations?" Sheets and Funk (1922) state that there was no difference in regard to growth and incidence of xerophthalmia in rats as the result of treatment with ultra-violet rays. They fed rats a diet deficient in vitamin A and exposed them daily to ultra-violet rays for 3 to 5 minutes. The rays prevented rickets and promoted growth, but did not prevent xerophthalmia.

Hume (1922) states that irradiation with the quartz mercury vapor lamp can prolong the growth of rats on a diet free or almost free from vitamin A, but attempts to relieve symptoms of vitamin A deficiency of animals which had been on a diet over 90 days failed. She concludes that there is an inter-relation of light and vitamin A, but no photosynthesis of the vitamin. Goldblatt and Soames (1922) concluded that rats' growth is

stimulated by irradiation with the mercury vapor lamp when the diet is deficient in vitamin A, but irradiation is not a substitute for the vitamin. Powers, Park and Simmonds (1923) found that sunlight delays the onset of xerophthalmia in rats, but they develop the disease quickly when they are taken indoors.

Vitamin Requirement of Different Species.—Experimental studies have led to the conclusion that not all species of animals have the same requirements for vitamin A. Guerrero and Conception (1920) produced xerophthalmia in fowls fed polished rice. Steenbock, Nelson and Hart (1921) found that within a period of 94 days ophthalmia developed in 3 out of 5 dogs on a diet lacking vitamin A. Other dogs given cod liver oil remained healthy.

Nelson, Lamb and Heller (1922) fed rabbits a diet deficient in vitamins A and C. One female gave birth to 5 young after 3 months on the diet; soon afterward she developed xerophthalmia. Two young died before their eyes were opened. A third died of ophthalmia when 2 weeks old, and the remaining two showed signs of it. Butter fat was then added to the mother's diet. In a few days the eyes of the mother were nearly well. The eyes of one of the young ones improved rapidly, and the animal doubled its weight in 13 days. This is direct evidence that vitamin A can be transferred promptly to the milk. Their results seem to indicate that the rabbit is immune to scurvy. Boock and Travan (1922) observed keratomalacia in guinea pigs confined to a diet deficient in vitamin A, and were able to cure it by giving cod liver oil. Sugiura and Benedict (1922) found that vitamin A is not essential for pigeons.

Emmett and Peacock (1922-23) found young chicks require vitamin A, but both young and mature pigeons require little, or none of it. The chick and the pigeon require no vitamin C. They state that the vitamin B requirement of the rat is less than that of the chick. Birds on a low A diet showed accumulation of urates. They also noted leg weakness in chicks on a diet deficient in vitamin A. Plimmer and Rosedale (1923) state that birds do not require vitamin C.

Beach (1923) fed fowls a ration which caused the development of a condition which would ordinarily be diagnosed as avian diphtheria or roup. The disease was not infectious and could be controlled by cod liver oil or green grass. He described xerophthalmia and the accumulation of crystal urates in the kidneys in poultry as a result of lack of A. The kidneys are

usually pale and are marked with a network of fine lines which are urate-filled tubules. He noted the deposit of white material, probably urates, on the liver and other organs. Hart, Steenbock and co-workers (1924) kept 2 groups of chicks on the same diet except that one had white corn and the other yellow corn, 97 per cent, supplemented with calcium carbonate 2 per cent., and sodium chloride 1 per cent. The birds were given skim milk ad libitum. The two groups were exposed to sunlight daily. The chicks fed white corn (deficient in vitamin A) did not respond to sunlight treatment, whereas those receiving yellow corn plus sunlight developed into beautiful birds.

According to Wolbach and Howe (1928) in man, and in the rat, a specific effect of vitamin A deficiency is seen in epithelial structures and results in the substitution of stratified keratinizing epithelium for normal epithelium in the respiratory tract, alimentary tract, eyes, parocular glands, and genito-urinary tract. The demonstration of a similar pathologic process in a strictly herbivorous animal, the guinea pig, supports the idea that the epithelial change is the expression of a fundamental physiologic requirement. It is obvious that these workers have opened up a most interesting field of research.

Methods for the Biological Assay of Foods for Vitamin A. —The first characteristic symptom of vitamin A deficiency to be noted was xerophthalmia. Recovery of the eyes of such animals and subsequent growth was the earliest test employed in the biological assay. While this test gives fairly good qualitative results, Hume and Smith (1928) who have studied most carefully the conditions under which most satisfactory results are to be obtained, stress the importance of adding vitamin A from the beginning of the experiment and of extending the observations over a long period in the assay of foods because of the prevalence of infections in the curative type of test. Deficiency of vitamin A appears to lower resistance to several kinds of infections.

Macy, Outhouse, Long, and Graham (1927) have suggested that the failure of animals to grow after a period of stunting for vitamin A, on adding an abundance of the latter to the diet, is the result of infection, which generally follows under the test conditions. They likewise point out that the presence of keratinized epithelial cells in the vaginal smears of virgin rats is an early and reliable sign of vitamin A deficiency. They give in detail a procedure which is probably the most accurate one

available for making the assay. An interesting comparison of the results of the arsenic or the antimony trichloride color test and the biological method of assay for vitamin A in a series of oils has recently appeared (Lancet, 1928, 148).

Vitamin A Content of Various Oils.—The fact that fats and oils generally from vegetable substances do not contain vitamin A, and that certain animal fats, e.g., cod liver oil, are very rich in it as compared with others, stimulated much experimental work directed toward comparing quantitatively the content of this substance in various fats and oils.

Zilva and Drummond (1921) found coal fish oil to be less effective than cod liver oil as a source of vitamin A. Pollock oil, although not so active as cod liver oil and coal fish oil, showed a high A content. Zilva and Miura (1921) found cod liver oil to be many times richer in A than butter. Drummond and Zilva (1922) described the various methods in effect for the preparation of cod liver oil in Norway. They studied oils prepared by different methods and found wide variations in vitamin A content in liver oils.

Zilva and Drummond (1922) found soft and hard roe of the cod rich in A; it was present in oil of ling, skate, plaice, and pollock. Zilva and co-workers (1924) studied the vitamin A content of cod liver oils taken from fish of different ages, and at different times in the sexual cycle, but found no influence of these factors. Among South African oils, Delf (1924) found sesame, mafureir and castor oils are deficient in vitamin A. Seal, whale and blubber oil of the sperm whale all contain it.

Holmes (1923) states that liver oils of emaciated cod are highly potent in vitamin A but are not so rich as the liver oils of plump cod. He (1924) found no difference in vitamin A content in oil from fresh livers and from livers stored at low temperature out of contact with air for six months to one year.

It will be seen that vitamin A is synthesized by plants and is associated in a remarkable way with yellow pigmentation. It is not synthesized by animals generally, but appears to be by pigeons. It is easily destroyed by oxidation, but in the absence of oxygen is fairly stable at somewhat elevated temperatures. In animal products it appears to be distributed fairly abundantly without association with yellow pigment as in pig liver, ghee, etc.

The results of studies directed toward discovering the chemical nature of vitamin A have led to great confusion. Certain data

would convince us that it is a sterol (Takahashi); other data appear to indicate that it can be formed from an inactive mother substance associated with cholesterol by the action of ultraviolet rays analogously to vitamin D (Drummond and coworkers); still other data seem to demonstrate that pure carotin or some substance accompanying it in ordinary methods of purification is the vitamin. Only further researches can clarify this important problem.

CHAPTER XIII

THE DIETARY DEFICIENCIES DISEASES—VITAMIN B

Beri-beri and Polyneuritis

Beri-Beri.—Beri-beri is a disease of great antiquity, which was long confined to Japan, the Dutch East Indies, Malay Peninsula, Philippine Islands, India, and Southern China. Cuerva (1923) says that the first information about the disease dates from about 2600 B.C., when it was already known to the Chinese. The next mention of it is in the second century A.D., when a Japanese physician described beri-beri as kak-ke, a name still used. It was at one time believed to be a tropical disease, but this view has been disproved by its frequent occurrences in other places, especially in the subarctic regions, notably Newfoundland, Labrador and Norway. It is due to faulty nutrition and will occur in any part of the world if man restricts his diet to certain foods. Vedder (1913) in his book "Beri-beri" cites several instances of its occurrence in the United States. According to him, Putman and Birge reported it in 1890 among New England fisherman; 71 cases were reported in an insane asylum in Alabama in 1895, of which 21 were fatal; it was also reported in 1907 among the patients in the insane asylum at Little Rock, Arkansas, and in a similar institution in Austin, Texas. A considerable number of cases were reported among the Japanese and Chinese laborers in California in 1909. In 1914 there was an outbreak in the jail at Elizabeth, N. J. All of these cases were due to poor diet.

Symptoms of Beri-beri.—Beri-beri is a form of peripheral neuritis, the nerves of motion and sensation being chiefly affected. Its incipiency is manifested by fatigue and depression, numbness and stiffness of the legs, and more or less edema of the ankles and face. It occurs in two forms, the wet and the dry. In the dry type, wasting, anesthesia and paralysis are the chief manifestations. The most marked symptom in the wet type is edema, which is sometimes excessive, affecting the trunk, limbs

and extremities. In both forms there is generally tenderness of the calf muscles and a tingling or burning sensation in the feet, legs and arms. Fernando (1924) says that laryngeal symptoms in the form of hoarseness and often aphonia have been observed in both infants and adults. Aphonia is one of the most characteristic symptoms of infantile beri-beri in the later stages of the disease. In adults it is not so frequent, but occurs occasionally. Vedder (1913) says of aphonia in beri-beri, "The speaking voice is lost and the patient can only whisper." Bontios (1642, quoted from Fernando) said: "While suffering from this disease the sound of my voice was so feeble for a whole month that those sitting next to me could not understand me." Vedder believes that aphonia in this disease is "probably due to degeneration of the pnemogostric nerves, thus causing paralysis of the laryngeal muscles." The mortality in this disease is usually high.

Theories Concerning Etiology of Beri-beri.—Since polished rice is poor in protein, fat and phosphorus, the prevalence of beri-beri among rice-eating peoples has been attributed to the low protein content of the diet, to fat starvation, to starvation for organic forms of phosphorus, or to intoxication caused by a poison assumed to be present in rice under unfavorable conditions. Climate, arsenical poisoning and specific infection have been proposed as etiological factors for the disease. Many now believe that beri-beri is the sequel to specific starvation for vitamin B. The history of the study of this disease by various investigators forms a chapter of absorbing interest in preventive medicine.

Study of Human Beri-beri by Takaki.—The most notable among the early investigations relating to the etiology of beri-beri is the work of Takaki (1887), Director-General of the Japanese Navy. About the year 1880 the disease became a scourge among Japanese sailors. Between 1878 and 1883 the average number of cases treated medically in the navy was 323.5 per 1,000 per year.

The principal components of the diet of the sailors were polished rice and fish, the remainder being derived from a number of vegetables. Takaki was impressed with the differences in the diet and in the health of the British sailors as compared with those of Japan, and suspected that beri-beri might be due to lack of protein. He commissioned a training ship to make a nine months' cruise, giving the common sailors a diet containing 91 gm. of protein per day. The better fed officers did not suffer

from the disease, whereas the men were afflicted. There were 169 cases of beri-beri among 276 men during this cruise.

Another ship was then commissioned to make a similar voyage with the protein raised to 155 gm. per man per day. Among the crew of 276 men there were but 14 cases of beri-beri. This experience led to an improvement in the sailors' diet, and to the disappearance of beri-beri from the service. More meat and vegetables were added to the diet and rice was replaced in part by wheat or barley. One and one-quarter pints of milk a day were furnished each man. Condensed milk was employed. The condensation of milk on a commercial scale began in England in 1856. Not only did beri-beri become rare, but also diseases of the digestive tract and of the respiratory system became less prevalent and diseases of the eyes were diminished to half the former incidence. From the eye conditions reported, it seems probable that the men were suffering from lack of vitamin A as well as vitamin B. The Japanese officials believed the increased meat consumption was responsible for the decrease in beri-beri.

For many years this experience of the Japanese navy was regarded as a demonstration of the importance of a protein-rich diet. That this was not the cause of the freedom of the sailors from beri-beri is clear from the history of the disease among the crews of Norwegian vessels. In the invigorating climate of the northern seas, beri-beri suddenly became common among Norwegian sailors after 1894, whereas before it had been unknown. Before 1894 their diet consisted of bread made from rye flour, milled without the removal of the germ, peas and salted meat, chiefly fat pork. After that year it consisted of white bread made from bolted wheat flour, peas, together with salt pork once or twice a week, preserved meat and fish three or four times a week, and dry fish once a week. The addition of meat and fish in liberal amounts, although increasing the protein consumption, was nevertheless followed by the sudden appearance of beri-beri, a new disease in that part of the world. Holst (1911) tells of one sea captain who refused to eat white bread, and provided for his own table a supply of the customary rye flour bread. When beri-beri attacked his crew he was able for a time to relieve his men by sharing his own brown bread with them.

Classic Experiments of Eijkman.—In 1889 Eijkman, Director of the Hygienic Laboratory at Batavia (Dutch East Indies),

observed a disease among the fowls kept at his laboratory, the symptoms of which were very much like beri-beri and the pathological anatomy showed the same degeneration of nerves. By several series of experiments he showed that this polyneuritis gallinarum occurred when the fowls were fed exclusively on polished rice, so-called white rice, in which the pericarp and the germ or embryo (called together silverskin) were wanting. The disease could be cured by adding silverskin to the white rice (1897).

Eijkman's first interpretation of the experiments was that the silverskin of the rice kernel contained some substance which neutralized the injurious influence of the starch-rich diet. To quote: "Aus den diesbezüglichen Versuchen zog ich den Schluss dass in den Silberhaütchen wahrscheinlich ein Stoff oder Stoffe vorhanden sind, wodurch der schädigende Influss der starkehaltigen Nahrung neutralisirt wird."

Eijkman found climate to be a negligible factor in the etiology of the disease. In the large cities of the East Indies (Batavia, Soeralaja), 66.7 per cent. of the people suffered from beri-beri, as against 30.9 per cent. among mountain dwellers. The greater number of the first group ate milled rice. Prisoners fed principally on milled rice showed an incidence of 80 per cent. near the sea, as compared with 62 per cent. in the mountains. In one prison 5.8 per cent. of the inmates suffered from beri-beri when supplied with polished rice, but the disease disappeared when undermilled rice was substituted.

Eijkman also proved that feeding exclusively with other starch-rich foods, such as tapioca, ambon sago and sago of the arenga-palm (Arenga saccharifera) also produced polyneuritis gallinarum. Potato meal was an exception, since with this food paralysis did not occur. He further showed that meat possessed some curative value and was to a certain extent a prophylactic against polyneuritis gallinarum. Eijkman concluded that faulty diet caused polyneuritis gallinarum. He came to the conclusion that the protective substance which was present in the silverskin (pericarp and germ) was wanting in such faulty diets and that it was a normal constituent of the animal organism.

Vorderman,* Sanitary Inspector of Java, in a conversation with Eijkman, expressed the belief that in his experience in several native prisons in Java where white polished rice (rice without silverskin) was provided, beri-beri occurred among the

* Personal communication from Dr. Hulshoff-Pol.

inmates, whereas he believed the disease had not occurred in those places where they were supplied with red rice (rice with silverskin). Eijkman recognized the importance of this communication, since it suggested the possibility of instituting experiments to show whether rice with silverskin prevented beri-beri. He induced the government to direct Vorderman to examine the effect of rice-feeding in connection with the prevention of beri-beri. Vorderman's report published in 1397 proved that among 96,530 native prisoners, three-quarters of whose diet consisted of unpolished rice, only 9 cases of beri-beri occurred (0.009 per cent.), whereas among 150,226 native prisoners who took white rice, or rice without silverskin, there were 420 cases (2.79 per cent.).

Although Vorderman's report showed clearly the advantages of the silverskin, it provoked criticism since only observations and no conclusive experiments had been made. Hulshoff-Pol says: "As often happens in such cases, criticism went much too far, people overlooking the good results of the work and emphasizing the weak points of it only." The physicians in the Dutch Indies were convinced that the possibility of an infection was not excluded, so Vorderman's views were not accepted and his ideas were applied in only a few prisons. Eijkman returned to Holland in 1898 and his researches were continued by Grijns.

Further experiments led Grijns to the conclusion that other food-stuffs had an influence on the origin and the cure of polyneuritis gallinarum. He proved that a bean "katjang hidjoe" had a strongly prophylactic and curative effect on fowls. Grijns further worked out the idea of Eijkman that the silverskin contained a substance which prevents and cures beri-beri. He called it a "deficiency" disease, since it was due to a deficiency of unknown substances. Grijns (1901) concluded that in several articles of natural food there exist substances which are indispensable since without them injury to the peripheral nervous system results. He stated that the "katjang-hidjoe" bean is rich, and polished rice poor in these substances.

Hulshoff-Pol says: "The official inquiry of Vorderman, not having the confidence of the physicians, who were quite wrong of course, most of them still being of the opinion that no prophylactic and curative remedy against beri-beri existed, the influence of these beans on beri-beri was examined by them." In 1901 Hulshoff-Pol made experiments at the lunatic asylum at Buitenzorg (Java). He added 150 gm. of boiled "katjang-hidjoe"

to the white rice, which he gave during 9 months to 78 lunatic natives; to 222 controlled lunatic natives he gave only white rice. None of the 78 persons who ate the beans had beri-beri, while no less than 68 of the 222 who did not eat them developed beri-beri. This experiment was repeated, but with a change of persons experimented upon, and again it was proved that the beans had a prophylactic effect. Their curative power became even more evident when the patients who had beri-beri because of an exclusive diet of white rice, were completely cured when each was given 150 gm. of "katjang-hidjoe" daily.

Hulshoff-Pol's researches were not accepted by the physicians in the Dutch Indies until 1907, when he succeeded in curing human beri-beri with a decoction of beans. Since he had separated a crystalline material from the decoction, the Government ordered an official inquiry concerning the value of the beans as a remedy for beri-beri. This research, executed by Kievit de Jonge, proved that the beans were curative. There have been no experiments on humans with the crystalline material (X-acid), but it has been shown that it will cure fowls suffering from polyneuritis gallinarum. In 1910 Fraser and Stanton confirmed the studies of Hulshoff-Pol.

Laurent (1899) attributed beri-beri to a deficiency of fat in the diet. He thought the beneficial effects of meat in the diet of Japanese sailors was due to its fat content. Montgomery Smith (1898) supported the infection hypothesis. He cited the ship *Lodestar* as having a bad reputation for beri-beri during successive years while the southwest monsoon blew.

Sir Ronald Ross (1901) believed that beri-beri was due to arsenical poisoning. Symptoms of peripheral neuritis have been observed in persons who drank beer containing arsenic.

Manson's Studies on Beri-beri.—Manson's studies (1901) convinced him that the disease was due to a toxin elaborated by micro-organisms growing outside the body. He suggested that improper handling of rice led to its infection and the development of a toxin. He opposed the theory that meat was protective against beri-beri, and cited the experience of the Singapore jail, where in 1898-99, there was an ordinary and a penal diet: the former contained wheat flour and fresh meat, the latter contained no meat. The greater number of cases of beri-beri occurred among those inmates of the prison having meat in their diet. McLeod (cited by Manson) mentions a case where beri-beri attacked the officers on shipboard, but not the common

sailors. The former had luxuries such as haddies, clams, oysters, lobster, and salmon. It was evident that lack of protein could not have been the cause of the outbreak.

Manson also cited the observation of Hirota, who saw 52 cases of the disease in nursing infants whose mothers were suffering from beri-beri. When the infants were weaned and fed condensed or fresh milk they recovered promptly, whereas five not so treated died. Manson argued that a child acquiring a micro-organism disease from the mother would not recover so rapidly, but that one intoxicated by poisonous milk would be expected to do so.

Is Beri-beri an Infectious Disease?—A most interesting paper on beri-beri has recently appeared from the Department of Hygiene, The Government University of Medicine of Chiba, Japan. Matsumura, Kakinuma, and co-workers (1929) believe it is an infectious disease and that a diet of polished rice is merely a predisposing factor. From their experimental work on pigeons they concluded that the chief causative agent in experimental beri-beri is an organism apparently closely related to the colon bacillus, and which they have named Bacillus beri-beri. They propose to classify the disease reproduced experimentally into two rather distinct types: the spasmodic paralytic form and the paralytic non-spasmodic form. The first type responds well to treatment with vitamin B, whereas with the second type injection of vitamin B is of uncertain value. Bacillus beri-beri was isolated from the feces of a considerable number of patients with beri-beri; they found that normal persons and experimental animals free from this disease did not harbor this bacillus. When this organism is fed to experimental animals, it becomes implanted in the intestine and the specific symptoms follow. One of the most interesting points brought out in their paper is that the serum of experimentally infected birds, as well as the serum from human cases of beri-beri, contain potent specific agglutinins for the beri-beri bacillus.

Studies on Human Beri-beri.—Fletcher (1907, 1908), at the Kuala Lumpur Lunatic Asylum in the Federated Malay States, carried out an experiment on human subjects to determine the relation of rice to beri-beri. He divided the hospital into two sections, each containing nearly equal numbers of patients. To one group he supplied milled rice, and to the other "cured" rice. "Cured" rice is prepared by soaking unhusked kernels, or paddy, in water, heating the grains until they burst and then drying

them in the sun. This rice is husked and ground for use without polishing. The kernel, including the germ, was supplied to half the patients. The daily diet of all the inmates consisted of fresh meat, fresh fish, salt fish, curry stuffs, coconut oil, rice, and vegetables. The only difference in the diets of the two groups of patients was in the character of the rice.

The diets were continued for a year with the following results: Many in the group eating the milled rice developed beri-beri, whereas no cases developed in the group fed the steamed (cured) rice. Fletcher mingled some of the beri-beri patients with the well patients, and continued the experiments. The result was a demonstration that the disease was due to a diet consisting in great measure of meat, fish, and milled rice. We now know that the same result will follow the too free use of endosperm of any of the cereal grains as food.

Toxicity Theory.—One of the hypotheses as to the cause of beri-beri was that food-stuffs contain something toxic. Schaumann (1908) was unable to detect any poison which might have been produced as a metabolic product by a micro-organism. He found no toxin in the urine of patients. He suggested that beri-beri was due to starvation for certain organic phosphorus compounds.

Since the early experiments of Schaumann there had been a tendency to associate beri-beri with Barlow's disease. Kohlbrügge (1911) connected these diseases with unbalanced diets too rich in starch. He believed such diets favored the growth of fermentative bacteria which formed acids and tended to self-sterilization of the intestine, depressing or extinguishing the normal flora. He designated them "fermentation diseases." He postulated the theory that a diet containing free acids tended to inhibit the growth of injurious bacteria.

Schaumann (1914-15) demonstrated that dried codfish and pickled meats, heated at 120° C., produced polyneuritis in dogs, cats, rats, a goat, and an ape.

In the early experiments the special importance of the germ of seeds was not recognized. The cortical layer was regarded as the one of importance. McCollum and Davis (1915) in their study of the wheat kernel first called attention to the fact that the germ was rich in the substance curative for polyneuritis. They suggested that it was the loss of the germ rather than of the cortical layer which deprived rice of its protective power in beri-beri.

The view that beri-beri is due to deficiency of phosphorus in the diet was supported by Edie and Simpson (1911). Polished rice and refined cereal foods contain little phosphorus and this suggested the phosphorus starvation theory. In the light of later experimental studies, these seemingly irreconcilable observations have been made clear.

Schaumann's Activator.—Schaumann (1915) showed that pressed yeast, wheat bran and dried testicle protect animals against polyneuritis. Other observers added egg yolk, beef heart, etc., to the list. Schaumann supplemented milled rice with purified protein and salts, but these did not prevent the disease. The failure of these substances and of phosphates to protect against beri-beri, confirmed his belief that there was a protective substance in certain foods which he called "activator." He correlated the high content of organic phosphorus in rice polishings with its curative power. He believed this "activator," to be an organic compound of phosphorus. This view was recognized by the Far Eastern Association of Tropical Medicine. It adopted a resolution that rice containing less than 0.4 per cent. of phosphorus should be excluded from those countries in which rice formed the principal food. The view that beri-beri is caused by a lack of phosphorus compounds has been abandoned.

McCarrison (1924) insists that beri-beri is not strictly a disease due to vitamin deficiency. In country districts about Madras, India, the home pounding of rice is still the common practice, and beri-beri occurs among the users of unpolished rice in endemic areas. The disease is about ten times less common among users of this rice than among users of polished rice in these areas, and whereas in endemic areas of the Madras Presidency beri-beri sometimes appears when undermilled rice only is used, it never occurs in non-endemic areas.

McCarrison states that contrary to the usually accepted belief, Mohammedans in endemic areas of the Madras Presidency suffer more from beri-beri than Hindus, although the former have a much more varied diet, and the latter restrict themselves much more rigidly to a rice dietary. In regard to the matter of caste, Pariahs, who eat anything, suffer most from beri-beri in endemic areas of the Madras Presidency; native Christians, who have no food scruples, suffer next in order of incidence of the disease; Mohammedans, who eat most things, come next; and Hindus, the greatest rice-eaters of all, last in order of affliction by beri-beri. He thinks these facts almost force us to the conclusion

that there are factors concerned in the etiology of beri-beri in endemic areas—apart from the too exclusive use of a rice dietary—which are absent or of feeble potency in non-endemic areas.

Wet Beri-beri; War Edema; Hunger Swelling.—It has

FIG. 12.—Edema in a baby fed too largely on skim milk and cereal foods. This condition is frequently associated with incipient ophthalmia of dietary origin.—Courtesy of Dr. Bloch.

been mentioned that beri-beri occurs in two forms, the wet and the dry. Persons suffering from the former type are dropsical, whereas those afflicted with the latter are usually emaciated. Until recently there was no clue to the cause of this difference. A brief history of the observations leading to its explanation will be of interest.

Hirsch (1885) mentions edema in the legs, serous cavities and lungs in some cases of beri-beri. Several investigators mention edema in scurvy. Gibson and Conception (1916) consider hypertrophy of the heart and edema typical of infantile beri-beri; they mention edema in beri-beri in dogs and pigs. McCarrison (1919) reports edema in pigeons not only after starvation but also in those fed polished rice.

There are recorded many epidemics of edema; it is also called epidemic dropsy, war dropsy and wet beri-beri. It occurs during periods of food shortage which become severe enough to produce famine conditions. In the 1876-77 famine in India, nine-tenths of all deaths were due to dropsy, dysentery, diarrhea, and nutritional debility. The government attempted to operate a system of rationing by determining the calorie needs calculated on the basis of body weight; but this system proved a failure because those who appealed for relief were found to be either emaciated or apparently very fat. The latter appeared to be in no immediate danger of starvation, although in many cases they were in imminent danger of death, since they were not obese but dropsical. The contrast between one group of the population which was nothing but skin and bones, and another which was bloated, occurred in persons of all ages. The history and literature in this field are so confusing that it is impossible to say whether wet beri-beri, war edema and epidemic dropsy are one and the same disease. Further research will have to decide this matter.

Wagner (1887) reported an epidemic of edema among infants in Germany caused by feeding modified milk alone or with cereal. Patterson (1899) described cases of edema among starving Chinese; weed seeds and greens obtained from wild plants were their only food. In 1917 when the city of Mexico was under military rule for several months, and spinach and beets were virtually the only articles of food, many cases of dropsy were reported. Potter (1904 and 1912) described an epidemic dropsy among infants restricted to barley water, which was very low in protein, inorganic salts, and fat. Czerny and Keller (1906) speak of "Mehlnährschaden" which is malnutrition resulting from too much cereal in the diet. Such a diet is too rich in carbohydrate, too low in protein and fat, and has serious mineral and vitamin deficiencies.

Holst (1911) states that dropsy occurred during the Crimean War when there was an epidemic of scurvy, and that dropsy

occurs every year on French fishing vessels off Newfoundland. Edema was common among prisoners in England and in America during the first half of the nineteenth century. This happened before prisoners received the humane treatment and moderately satisfactory diet now provided. Budzynski and Chelchowski (1916) described "hunger swelling" in Poland during the World War.

The cases recorded point to a low protein dietary as the condition favoring the development of the disease. Experimental data seem to prove that protein starvation is the specific cause, but a water-rich food especially promotes the development of edema. Denton and Kohman (1918) restricted young rats to a diet of carrots and found that a large percentage of them suffered from edema. Kohman (1920) fed carrots supplemented with purified food substances, and found that edema could be produced only in animals confined to an inadequate protein intake and given somewhat excessive amounts of water with their food. Maver (1920) refers to the occurrence of edema in horses and oxen which work about sugar factories and subsist upon sugar cane. Cattle fed too liberally on distiller's mash or beet pulp become dropsical.

Hoelzel (1928) calls attention to the fact that during his 20 years of personal experimentation with fasting, edema was frequently manifested. It often occurred after periods of undernutrition but was most prominent after fasting. He believes that the European studies of "war edema" made it clear that nutritional factors and not impairment of the circulation or of kidney function were responsible. He postulates an interesting theory on the relation of nutritional edema to the common cold.

Pathology of Nutritional Edema.—Weltmann (1921) says in regard to the pathology of famine edema: "Three cases of the disease showed symptoms indicating pancreatic insufficiency as diarrhea, steatorrhea and kreatorrhea. The duodenal juice showed almost no tryptic activity and also a marked reduction in lypolytic and amylolytic activity. Autopsy showed marked atrophy of the pancreas."

Ashford (1922) reports cases of beri-beri in the garrison at San Juan, Porto Rico. The pathological condition was a form of beri-beri complicated by glandular deficiency. This, he believes, was induced by protein deficiency in the food. The ration furnished sufficient vitamin B, but contained too much rice and fat and too little fresh meat and vegetables. The natural food habits

of the native soldiers led them to eat enormous quantities of polished rice. Mann (1920 and 1924) described a condition among prisoners in Haiti resembling beri-beri. There is a wet and a dry form of this disease. He mentions the following features of the disease which seem to differentiate it from true beri-beri:

Beri-beri	Edema Disease of Haiti
Cardiac hypertrophy	No case of cardiac hypertrophy seen in nearly 200 autopsies. The heart is usually reduced in size.
Palpitation and rapid pulse rate.	Palpitation not marked; slow pulse rate, usually around 50 per minute, as low as 28 per minute recorded.
Motor paralysis sometimes present.	Only one case of paralysis was discovered.
Vomiting sometimes present.	Vomiting never detected.
Fever sometimes present.	Fever usually absent. Temperature subnormal.
Loss of appetite.	Appetite increased and perverted.
Symptoms required longer period to develop.	Symptoms develop more rapidly, often within 3 weeks after exposure to the etiological factor, and as early as 10 days.
Steppage gate.	Not present.

Dilatation of the right side of the heart is reported for both scurvy and beri-beri (Hess, 1917). Vedder (1916) points out striking similarity in the nervous symptoms observed in scurvy, beri-beri and pellagra, and notes that there are certain resemblances in their symptomatology and pathology.

Kobayashi (1923) states that in contrast to nephritis, and nephrosis, the edematous fluid contained as little as 0.2 or even 0.1 per cent. of albumin. The composition of the blood was nearly normal.

Vedder (1923 and again in 1928) states that there is a strong possibility that the two forms of beri-beri may be caused by lack of two vitamins; dry beri-beri being caused by a deficiency of the anti-neuritic vitamin, while wet beri-beri, epidemic dropsy and prison dropsy are due to a deficiency of a second as yet unnamed vitamin.

It seems plausible that in wet beri-beri, protein starvation is superimposed upon a deficiency of vitamin B. Wet beri-beri may be due to a double, and dry beri-beri to a single deficiency. A diet which produces beri-beri is so poor in quality with respect to several factors that the syndrome is usually a complicated one. This explains the tendency of students of the diseases of

dietary origin to emphasize the points of similarity of the two deficiency diseases, scurvy and beri-beri.

Pathology of Polyneuritis.—A new conception of the pathology of polyneuritis and other "deficiency" diseases was introduced by McCarrison (1919). The most striking manifestations of polyneuritis are the loss of the coördinating powers of the muscles. The onset of the disease is generally preceded by the bird sitting with ruffled feathers, and with the appearance of illness. There is progressive weakness, and when disturbed there is a tendency for many pigeons to be taken with convulsive seizures in which they turn "cart-wheels" backwards at intervals until they die. In the acute type of the disease many birds sit with the head greatly retracted. These symptoms generally led investigators to accept the view that the lesions in beri-beri were principally situated in the nervous system. His studies reveal that injury to the nerves is much less pronounced than injury to certain other tissues.

McCarrison observed functional and degenerative changes in every tissue of the body. The thymus, testicles, spleen, ovary, pancreas, heart, liver, kidneys, stomach, thyroid and brain underwent atrophy, the severity in his cases being in the order named. The adrenals suffered hypertrophy, and this was associated with a proportionate increase in the adrenalin content of the glands. Edema was invariably associated with hypertrophy of these glands, suggesting its relation to an excessive production of adrenalin. Inanition gave rise to adrenal hypertrophy and to atrophy of other organs, the brain excepted, similar to that observed in birds fed solely upon polished rice. Rice is deficient in all vitamins, protein and several inorganic elements. The gastric, intestinal, biliary and pancreatic disorders of birds fed only polished rice were more serious than the nerve lesions. A rice diet gave rise to congestive and atrophic changes in all the coats of the intestine, especially the duodenum; to lesions in its neuromuscular mechanism; to impairment of its digestive and assimilative functions, and to failure of its protective resources against bacterial infection. Guinea pigs restricted to a diet of oats and autoclaved milk developed lesions of the digestive tract analogous to those seen in pigeons fed polished rice. Owing to the multiple deficiencies of the rice diet, it is impossible to decide as to the specific effects of deficiency or lack of a single dietary component. It is interesting to note, however, that on a diet deficient in vitamin C., guinea pigs developed lesions com-

parable with those found in birds with diets faulty in several respects.

McCarrison (1919) fed pigeons a diet of polished rice, butter fat, and raw onions. This was deficient in the anti-neuritic substance and in mineral elements, especially calcium, phosphorus, sodium, chlorine, and potassium. The butter fat supplied vitamin A and the onions vitamin C. Typical symptoms of polyneuritis appeared, showing that onion contains little vitamin B. Atrophy of the bowel in these birds was usually less marked than in birds on the rice diet alone. Congestion was less prominent; atrophy of the myenteron and of the elements of the mucosa were often comparatively slight. Two of twelve birds examined had bacterial infection of the bowel.

In a number of the polyneuritic birds the blood was infected by B. suipestifer, B. pyocyaneus, and another organism not identified. McCarrison pointed out that systemic infection was facilitated by the presence of the pathological processes existing in the intestine as the result of dietary deficiencies. Invasion was favored by the impaired production of digestive juices, by malnutrition of the secretory cells owing to the congestion of the mucous membrane, by the increased leucocytic traffic in micro-organisms between the mucous membrane of the intestine and the blood, by the greater opportunity which the debilitated mucous membrane provided for the growth of micro-organisms on its surface and within its substance, and to actual breaches of continuity in the walls of the bowel itself. The imperfect digestion in the upper part of the tract yielded a medium favorable for the growth of bacteria. Unwholesome products tended to further debilitate the mucosa, and to increase the prospects of invasion by micro-organisms. Infection of the blood from the bowel under such conditions would be expected and was repeatedly demonstrated by aerobic cultures of heart blood.

In guinea pigs restricted for a time to a diet of oats and autoclaved milk, McCarrison also observed localized destructive changes in the mucous and underlying coats of the stomach and duodenum. These changes were manifest before the animals had developed any of the characteristic naked eye appearances of scurvy, and he regarded them as pre-scorbutic.

Injury of Nervous System Less Than of Other Tissues.—McCarrison further pointed out that the nervous symptoms of polyneuritic birds may be ameliorated or recovered from and yet the birds may die as the result of the gastro-intestinal lesions.

He emphasized that the rapid disappearance of the nervous symptoms indicated that of all the tissues, the nerves are least affected as regards organic lesions by vitamin deficiency. More serious damage resulted to the intestine, and recovery was correspondingly slow. The remarkably rapid disappearance of convulsive seizures, of astasia and of cerebellar symptoms, indicated clearly that specific fasting for the anti-neuritic substance was in some manner intimately concerned with the functioning of the nervous system. This may be an activating or energizing action on the nerve cells, or a necessary condition for the completion of the nervous current. In the absence of this substance the nerve cells are incapable of normal activity, and when it is provided normal functioning is reëstablished with dramatic suddenness. McCarrison accepts the view, expressed by Bayliss, that the function of the anti-neuritic substances is that of a catalyst.

McCarrison (1922) suggests that subminimal provision of vitamins results in marked impairment of the neuro-muscular control of the intestine. Consequent upon this there is failure of the latter to transport its contents in a normal manner, due to impairment of the assimilative, secretory and protective functions. He suggested lack of sufficient vitamins as an etiological factor in certain pathological states in human subjects which appear to be the analogue of these experimentally induced states in animals.

He reported that pigeons fed polished rice frequently suffer from diarrhea, and this symptom of vitamin deficiency disappears on administering alcoholic extracts of egg yolk. Diarrhea and colitis are almost constant precursors or concomitants of "war edema." Subminimal dietetic deficiency of certain types may lead to a state of chronic gastro-intestinal catarrh in which the intestine produces abnormal amounts of mucous. Mucous disease is very common among European children in India who are fed largely upon sterilized milk, artificial foods, white bread, polished rice, butter, overcooked vegetables, and excessive quantities of sugar. Therapeutic experience has shown that this malady yields to a rationally selected dietary. The mucous stools, the diarrhea, the marked irritability, and the unhealthy appetite rapidly disappear on such a regimen, as do also the "night terrors" from which such children frequently suffer (McCarrison).

Celiac Disease.—In the category of syndromes caused in children by feeding insufficient diets, McCarrison also ventures to

suggest "celiac disease" described by Still (1918). It is thus characterized: "Absent in breast-fed children, its onset between the age of nine months and two years, the diarrhea which so frequently precedes it, the cessation of growth, the ill-formed, pale, 'oatmeal stools,' the frequent association of scorbutic symptoms, the abdominal distention, the afebrile nature of the malady, the diminished size of the liver, the blood changes, the occurrence of edema, the thin bones, the muscular feebleness—all these find their counterpart in pigeons fed on an exclusive dietary of autoclaved milled rice."

McCarrison also draws a parallel between the conditions observed in animals on faulty diets and the anatomical factors seen in chronic intestinal stasis. Keith (1918) regards defective action of the abdominal musculature, and a lesion of the neuro-muscular system of the intestine, as the two factors of primary importance in the causation of intestinal stasis.

Defects in McCarrison's Diets.—McCarrison is hardly justified in speaking so unqualifiedly of "vitamin deficiency" as the cause of the lesions observed. The extensive studies on rickets have shown clearly the serious consequences of deprivation of certain mineral elements, notably calcium and phosphorus. McCarrison's diets were almost without exception deficient in several inorganic elements, including calcium, phosphorus, sodium and chlorine, and contained an excess of acid over basic radicals. Deprivation of chlorine quickly leads to inability of the stomach to secrete hydrochloric acid, and this alone would seriously interfere with digestion, and with the suppression of bacterial growth in the alimentary tract of which the normal intestine is capable in some degree. Diets such as polished rice; rice and butter; or rice, butter and onion, obviously so deficient in mineral salts, might well lead to a state of depletion of the alkali reserve in the blood. Such deficient diets may account for the air hunger which he attributed to acidosis brought about by acid fermentation resulting from a diet too largely derived from carbohydrate. The conditions which he described, however, are induced by dietaries of a type which is at present common in Europe and in America. Aside from details which may require revision, the conclusions of McCarrison represent an important advance in our understanding of the relation between faulty nutrition and physical deterioration.

Megaw and Banerji (1923) reported 12 cases of beri-beri which occurred in two well-to-do and highly cultured families consist-

ing of 13 members, ranging in age from 8 to 80 years. The most striking manifestations of the disease were gastro-intestinal symptoms which occurred in 10 out of 12, diarrhea in 8, and dysentery in 1.

Does Beri-beri Occur Uncomplicated with Scurvy?—In the light of the description which has been given of the dietary properties of natural food-stuffs and the products made from them, it is instructive to study the deficiencies of typical diets to which many cases of this deficiency disease have been attributed. In the Burma prison there was an epidemic of scurvy with the following dietary (1914, Bull. of Tropical Diseases):

	Oz. daily		Oz. daily
Rice (husked)	24	Condiments	0.125
Beans	4	Fish paste	0.5
Vegetables (kinds not specified)	10	Salt	0.25
Oil (vegetable)	0.5		

Fleming (1910) reported epidemics of scurvy in mines in Southern Rhodesia where the men were given the following foods:

Mealie meal (milled maize)	2	lb. daily
Meat	1	lb. weekly
Beans	2	lb. weekly
Monkey nuts	1.52	lb. weekly

Chamberlain (1915) reported that among 5,000 Philippine Scouts there were always from 100 to 600 incapacitated with beri-beri. Their diet consisted of the following foods:

	Oz. daily
Beef	12
White flour	8
Potatoes or onions	8
Polished rice	20

On changing the diet by substituting 16 ounces of unpolished rice and 1.6 ounces of beans for the 20 ounces of polished rice, and including 20 ounces of sweet potatoes, by the end of 1910 the number of cases of beri-beri had decreased to 50; the following year there were 3 cases; in 1912 but 2, and no cases in 1913.

Hehir (1919) reports that during the siege of Kutel-Amara from December to April, 1916, the British troops used white flour biscuits, tinned meats and horse flesh as food. Many cases of beri-beri were reported. Gastro-intestinal symptoms and marked distension of the abdomen were present in many cases. No scurvy

developed. The Indian troops used dal and either atta or barley flour, but refused to eat fresh meat. They were free from beri-beri but suffered severely from scurvy. The British troops were protected from scurvy by the vitamin C of the fresh meat, and the Indian troops from beri-beri by the nature of their cereal diet.

Voegtlin (1920) selected the following diet (Diet A) for 100 patients in the Pellagra Hospital at Spartanburg, S. C., who were suffering from mild and uncomplicated pellagra. It is typical of the food supply of numerous homes where cases of pellagra are observed. If the cabbage were cooked it is not clear why these patients did not also suffer from scurvy. The small amount of vitamin C in cooked potatoes may have protected them.

DIET A

Wheat bread	300 gm.	Pork	50 gm.
Butter	30 "	Potatoes	150 "
Cabbage	100 "	Prunes	30 "
Corn meal	50 "	Turnip tops	100 "
Ham	25 "	Sugar	40 "
Hominy	75 "	Milk	40 "
Corn Syrup	30 "		

Patients restricted to Diet A failed to improve or grew noticeably worse. They were later changed to Diet B, a similar diet, but one which contained more milk, meat, and eggs. Most cases showed definite improvement in 2 weeks, and in 2 months or more were pronounced cured, except in a few more severe and advanced cases. The therapeutic value of the detetic treatment was very spectacular.

It is impossible to say why the Burma Prison diet produced scurvy rather than pellagra. It is possible that the small amount of beans contained enough vitamin G to protect against it. The Rhodesia miners' diet contained meat which provided the anti-pellagra vitamin G and so produced scurvy from lack of C. The diet reported by Chamberlain caused beri-beri rather than pellagra, since meat furnished G, but the diet was deficient in B. The possibility is not excluded that pellagra cannot be produced in those species which have been studied because of the presence of synthetic powers not possessed by man.

The diets described above have another meaning: on any of them one would suffer from several deficiencies, and where scurvy was observed it seems evident that there must also have been a serious shortage of vitamin B and consequently a tendency for

symptoms of beri-beri to develop. Furthermore, the diets listed are all decidedly deficient in one or more inorganic elements, notably calcium. In those diets poor in meat there was also a shortage of phosphorus as measured by the requirements of the rat.

It seems probable, therefore, that in many instances where one of the diseases, beri-beri or scurvy, is diagnosed, the other exists in a latent stage. This would account for the points of similarity in the manifestations of the two diseases as they have been described. It also raises the question whether the specific effects of lack of vitamin C or of vitamin B, the remaining components of the diet being of optimum quality, can be said to be fully known in the human subject.

The addition to a diet producing beri-beri in the Orient of some article of food which will prevent beri-beri, will in many instances not correct the inorganic faults. From the standpoint of public health, therefore, it is not sufficient to supply vitamin B (e.g., tiki-tiki extract). This may be done and yet the population may be poorly nourished. The infant mortality may be high because of the poor quality of the mother's milk, and adult members of the population may still early develop signs of senility. These statements are made on the basis of the assumption that the same physiological limitations with respect to nutrition which apply to animals apply also to man, and that these run parallel when the comparison of an animal with a man on the same diet is made on experiments covering equivalent fractions of the average span of life of each.

Normal Versus Optimum Nutrition.—There has been a tendency to assume that a diet which does not lead to clinically recognizable symptoms of malnutrition is an adequate one. Now that we understand more about the nutritive needs of the body and more about the properties of food-stuffs, we appreciate the existence of many grades of malnutrition. It is becoming constantly more evident that even a slight departure from the optimum in the composition of the food may lead to states of nutritional instability which become contributing factors to physical breakdown when hygienic factors are unfavorable, or when infectious processes are operating.

McCarrison conceives the nutrient principles in the class which now are called vitamins as "certain nuclear ingredients essential for the nutriment of the living nucleus. They are 'nuclear nourishers' without which multiplication of cells does not occur.

The term *nucleopast* (that which feeds the nucleus) might well be applied to them."

Exercise and Vitamin-Deficient Animals.—McCarrison says that human beri-beri is brought on more acutely by physical exertion. Yoshiue (1922) showed that avitaminosis brought about severe functional disturbances in the central and peripheral nervous system as well as in other organs. His exercised rats, without any appearance of muscular paralysis, died sooner than the others. Keith and Mitchell (1923) placed rats in cylindrical boxes making 13 revolutions per minute. The rats traveled at a rate of half a mile an hour with rest periods between the periods of activity. They exercised between 30 to 40 hours a week. Exercise distinctly hastened the appearance of the symptoms of vitamin A deficiency and hastened the death of young animals. They believe muscular exercise increases the demand for vitamin A.

Blacklock (1924) stated that "beri-beri is notoriously a disease which occurs in institutions and in places where a fixed diet and some degree of confinement are almost constantly present—for example, prisons, asylums, ships, etc." He believes "that lack of exercise accelerates and accentuates the ill effects of diets deficient in vitamin B."

It is now easy to understand how there could arise so many different views concerning the etiology of beri-beri. In themselves the observations were generally reliable, but it was impossible to interpret them because nobody understood what factors were necessary for an adequate diet, and the specific rôles they played. The existence of the vitamins, while hinted at by the prevailing views on scurvy, and by the early studies of beri-beri, were on the whole practically unappreciated. The recorded speculation regarding them was nebulous. As has been pointed out in Chapter III, the demonstration of the existence of such factors by crucial experiments on animals was a matter of great difficulty. The history of beri-beri serves as an example of the inability of pathologists or epidemiologists to solve the problem of the etiology of such a disease. It illustrates the value of fundamental chemical research directed toward determining the chemical items which can serve as the simplest adequate diet for man or animal.

There exist differences of opinion as to whether or not beri-beri is the result of vitamin B starvation alone; but this question cannot be decided on the basis of existing evidence. It also

remains to be established whether or not the rat and the pigeon tests for the anti-neuritic principle, actually determine the same substance. No experiments exist to demonstrate whether all the symptoms of beri-beri are the result of deficiency of the vitamin B alone.

There are pathological changes which are specific for each of the deficiency diseases as they relate to vitamin starvation. It is not certain that the syndrome caused by deprivation for any one of the vitamins has been adequately described. In some of the experimental evidence presented in this chapter it is apparent that faults other than a lack of a vitamin existed, and it is possible that these contributed to the changes produced. The lesions produced by deficiency of different vitamins in the diet are so distinct that there seems to be no doubt that the manner in which each affects metabolism is peculiar to itself.

CHAPTER XIV

ATTEMPTS TO ISOLATE VITAMIN B (THE ANTI-NEURITIC SUBSTANCE)*

In the preceding chapter the studies of Eijkman, Grijns, Hulshoff-Pol, Fraser and Stanton, Schaumann, and others have been recorded, in which they demonstrated the existence of the anti-neuritic principle and discovered certain of its properties.

Funk (1922) summarized the available knowledge of the chemical nature of the anti-neuritic vitamin up to 1911 as follows: (1) The substance is soluble in water, alcohol and acidified alcohol; (2) it is dialysable; (3) it is destroyed at 130° C.

Concentration of Vitamin B.—Osborne and Wakeman (1919) formulated a method for preparing an extremely potent preparation of vitamin B. By fractionally precipitating an aqueous extract of yeast with alcohol they secured an active fraction in which most of the vitamin was present with only a small proportion of inert material. At the outset they prevented protein and much inactive water-soluble material from entering the yeast extract by boiling the cells with acidified water. Inasmuch as the extract was comparatively free from the products of autolysis, the conditions for fractionation were simplified.

A water extract of brewers' yeast concentrated to a small volume was poured into sufficient strong alcohol to make the spirit content of the mixture approximately 52 per cent. by weight. The precipitate formed, known as fraction 1, was inactive. Therefore, the filtrate was reduced in volume, and was poured into alcohol until the concentration of the solution became 79 per cent. The precipitate which appeared was designated fraction II. The filtrate was now added to sufficient alcohol to bring the concentration of the mixture to 90 per cent. alcohol by weight. This precipitate was called fraction III. The filtrate from it was reduced to dryness and termed fraction IV. By feeding tests upon rats it was determined that most of the

* For a detailed discussion of this subject see Kruse and McCollum: Physiological Reviews, 9, 126 (1929).

vitamin originally present in the yeast was concentrated in fraction II. Thus, Osborne and Wakeman were successful in concentrating the greater part of the vitamin in about 6 per cent. of the dry yeast solids. That the active fraction was not free from mineral matter was shown by its ash content of 10.65 per cent. This figure, however, was considerably lower than that recorded for any inactive fraction.

Seidell's Attempts to Isolate the Vitamin.—Seidell (1916) announced that vitamin B was absorbed from autolyzed yeast by a particular variety of fullers' earth known as Lloyd's reagent. The vitamin-Lloyd's reagent combination was termed "activated solid." Within a short time Seidell and Williams (1916) learned that the anti-neuritic substance could be removed from the activated fullers' earth by extraction with sodium hydroxide or baryta solution. By further purifying the material yielded by this adsorption method, Seidell (1921) secured a relatively stable silver-vitamin compound. In this process the anti-neuritic substance was removed from the activated solid by baryta solution, and the active extract was clarified by lead acetate precipitation. The filtrate, freed from excess lead, was concentrated to a small volume under reduced pressure. Several crops of white, amorphous material separated, but all were inactive. The solution finally became a thick, viscous mass. When silver nitrate solution was added to this residue dissolved in water, a voluminous precipitate formed which was inactive. Addition of ammoniacal silver nitrate to the filtrate produced another precipitate, which contained a readily crystallizable inactive substance and the difficultly crystallizable vitamin compound. The filtrate therefrom was practically free from the anti-neuritic principle. After removal of the inactive crystalline compound from the precipitate, the vitamin was again thrown down as a silver compound with its anti-neuritic properties unimpaired.

The insoluble compound formed by silver nitrate precipitation was mainly adenine; while the inactive crystalline substance produced by ammoniacal silver nitrate precipitation was a base related to histidine, although it was neither histidine, histamine, nor carnosine (1921). By this time Osborne and Wakeman's method of fractional precipitation by alcohol had appeared in the literature; therefore, Seidell (1922) used this active fraction as the starting point for adsorption of the vitamin by fullers' earth. In this way the presence of adenine, one of the interfering

substances simultaneously adsorbed with the vitamin, was avoided.

After incorporation of this modification into his method, Seidell (1922) determined that approximately one-third of the solids of the extract from fullers' earth was precipitated as silver compounds, containing somewhat more than half of the anti-neuritic principle. He attributed the incomplete precipitation of the vitamin to the considerable solubility of its silver compound. Nitrogen determinations used as criteria of purity led him to believe that the vitamin fractions were mixtures of two or more analogous bases. The chemical similarity of such compounds was inferred from their common adsorbability on fullers' earth, their common extraction by aqueous alkali, and their common precipitation by ammoniacal silver nitrate. Seidell concluded that the anti-neuritic substance could not be advantageously separated from the other bases by silver precipitation.

While working with Bertrand, Seidell (1923) showed that somewhat more than 80 per cent. of the vitamin present in an extract from activated solid could be converted into a crude picrate. Fractionation of this product by recrystallization from 95 per cent. acetone yielded two crystalline picrates. The one, obtained from the acetone-insoluble portion of the crude picrate, consisted of pale yellow rods or prismatic crystals, melting at 202°. These were inactive. The other, derived from the acetone-soluble portion of the product, yielded aggregates of irregular-shaped crystalline flakes. In doses as low as 2 mg. daily these crystals protected rice-fed pigeons from loss of weight. Analyses of the two picrates gave results indicating an empirical formula of $C_6H_{18}O_2N_3OHC_6H_2(NO_2)_3$. Seidell suggested that the two compounds were very closely related; indeed that they might be tautomeric forms of the same compound. Later (1924) he remarked that the results from combustion analyses on the active picrate had not been sufficiently satisfactory for the calculation of a formula. At the same time he indicated that a portion of the nitrogen of the potent compound was present in the amino form. Finally Seidell was able to remove the picric acid from the vitamin picrate by means of nitron. The resulting free base retained its anti-neuritic properties (1925). The molecular weight of the base, determined by freezing point method, was 201; a combustion analysis gave results corresponding to the formula $C_8H_{15}O_3N_3$. One amino nitrogen group was present in the molecule.

Levene's Studies on Vitamin B.—Using the Osborne-Wakeman active fraction as a starting material, Levene and van der Hoeven (1924) further concentrated its anti-neuritic content by precipitation with barium hydroxide. Although the product obtained by this step was from five to ten times as potent as the initial fraction, the procedure was attended with a loss of about half of the vitamin originally present. They (1924) turned then to the use of various adsorbents, by which means they prepared extraordinarily active concentrates. From the behavior of Lloyd's reagent toward the vitamin they formulated a theory concerning the adsorbing mechanism of the former substance. The surface of the reagent acted as a salt of a weak acid, they said; and it was capable of a double exchange reaction with slight complications. They assumed from this fact that "its adsorbing power would be affected by the degree of ionization of the active surface of the adsorbent, and the degree of dissociation of the substance to be adsorbed." Since both conditions depended upon the hydrogen ion concentration of the medium, Levene and van der Hoeven postulated the existence of a certain pH for each substance when its adsorption was at an optimum. This theory was confirmed by their finding that the optimum adsorption of the vitamin upon Lloyd's reagent occurred at pH 4. The optimum extraction of the active principle took place at pH 9.

Extending their studies, they found that silica gel would adsorb the active material most effectively at pH 5. What was striking in this instance was the discovery that the vitamin could be removed from the gel not only by alkalies but also by acids. However, only a part of the anti-neuritic substance was extracted at pH 3; the larger portion was removed by pH 9. When material obtained by barium precipitation was submitted to this silica gel process, its activity was increased 200 to 400 times. At this point Levene and van der Hoeven introduced several new steps into their procedure, and rearranged their plan for concentrating the vitamin.

Under the revised process the anti-neuritic principle in the Osborne-Wakeman (1925) fraction of yeast was precipitated successively by basic lead acetate, and by barium hydroxide. The active material yielded by this treatment was adsorbed upon silica gel, and then was removed under the prescribed conditions. The final product contained 4.6 per cent. phosphorus and 15 per cent. nitrogen. It showed positive reactions for carbohydrates,

and was active upon rats in about 0.1 mg. substance. Levene and van der Hoeven pointed out that the co-adsorption or even introduction of mineral impurities, particularly phosphates, was a very disturbing factor in the purification of the vitamin.

When the active material obtained from lead-barium treatment was precipitated with absolute alcohol in a strongly acid medium, the troublesome inorganic salts were removed. Such a purified preparation furnished after silica adsorption a final product free from phosphorus. It contained only 4.2 per cent. nitrogen. However, its activity of 0.6 mgm. in a daily dose was much less than that of the phosphorized product. Apparently purification of the material by removal of inorganic salts was attained with considerable loss of activity, and the resulting product was not so effectively improved by silica adsorption as was the phosphorized material. Levene and van der Hoeven remarked that the phosphorus-containing impurity seemed essential for selective adsorption by silica.

Although the preparation derived from the foregoing method was extremely potent, it was a complex mixture. In addition, its further purification led to considerable loss in activity. Therefore, Levene and van der Hoeven (1925-1926) abandoned this process, and developed a more economical method of concentration. Adjustment of a solution of the Osborne-Wakeman fraction to pH 4 formed an inactive precipitate. The filtrate was deaminized by nitrous acid, and then was submitted to silica gel adsorption. The final product, removed from the adsorbent, was a complex mixture comprising carbohydrates, nitrogenous substances, and esters of phosphoric acid. It possessed no amino groups, but it contained sulfur in the thiol form. Levene and his associate remarked that the sulfur content had increased with purification, but they deemed it would be premature to assert whether the sulfur was a part of the active substance. An analysis of the final product gave the figures (calculated ash free): C = 41 per cent.; H = 6.7; N = 9.0; P = 16.5; total S = 3.9, thiol S = 2.0; and $NH_2 = 0$. This preparation was active for rats in daily doses of 0.08 mg.

Whereas Seidell had essayed to isolate the substance preventing polyneuritis and loss of weight in the pigeon, and Levene and van der Hoeven the vitamin inducing growth in the rat, Peters (1924) sought to separate the curative principle relieving head retraction in the pigeon. Peters termed this substance torulin, and stated that it might or might not be the same vitamin

with which Seidell, and Levene and van der Hoeven had dealt. In Peters' concentration of torulin, an aqueous extract of yeast was clarified by successive treatments with lead acetate, and with acid mercuric sulfate. The active substance in the filtrate was adsorbed upon norite, a birchwood charcoal; it was then removed from the adsorbent with 50 per cent. alcohol containing hydrochloric acid. This extract was active in daily doses of 3 mg. solid.

Peters' Method for Preparing Active Preparations.—Later Peters with Kinnersley (1925) extended the procedure of purifying torulin by introducing subsequent steps which involved precipitation of inert material by lead acetate, by dialyzed iron, and by ethyl alcohol; fractionation in an alcohol-ether mixture; and finally fractionation by silver salts. They obtained a yeast concentrate which was active in daily doses of 0.084 mg. When this method was applied to large scale production, complications arose in the pre-charcoal steps (1927). Accordingly, a treatment with baryta for removal of gums was inserted between the lead acetate and acid mercuric sulfate precipitations. Then the torulin was adsorbed upon norite at pH 7. Kinnersley and Peters stated that the curative substance was best removed from the charcoal adsorbent by two extractions: one with 0.1 normal hydrochloric acid at a pH acid to congo red, the other with acid 50 per cent. alcohol. The combined extracts, when subjected to alcoholic fractionation, yielded a product containing 15 to 25 per cent. nitrogen and showing activity in a daily dose of 0.15 to 0.30 mgm.

Jansen and Donath's Crystalline Preparation.—Jansen and Donath (1926), working in the same laboratory in which Eijkman made his fundamental discovery, announced the isolation of the anti-beri-beri vitamin in crystalline form. In their method the anti-neuritic substance in rice polishings extract was adsorbed at pH 5 upon acid clay, from which it was subsequently removed by baryta. When the clay extract was subjected to fractionation with silver nitrate-baryta, more than 50 per cent. of the vitamin was precipitated by these reagents at pH 6.5. The decomposed precipitate yielded a solution which was decolorized by norite without loss of the vitamin. This latter step is interesting in the light of Peters' use of the charcoal as an adsorbent for the anti-neuritic substance. The clear filtrate was treated with phosphotungstic acid; the resulting precipitate was decomposed;

and its solution was concentrated to dryness, and taken up in absolute alcohol.

Addition of platinic chloride precipitated practically all the vitamin and only one-fourth of the solid matter present in the alcoholic solution. The solution obtained from decomposition of the platinum precipitate contained one-fourth of the vitamin originally present in the polishings, and at least two other substances associated with the active principle. That this number of substances was present in the admixture became evident from an acetone-alcohol treatment, which yielded three fractions: the first was insoluble in absolute alcohol; the second was soluble in absolute alcohol, but insoluble in acetone (this contained the vitamin); the third was soluble in both absolute alcohol and acetone. By further purification of the second fraction the vitamin was obtained in crystalline form as a hydrochloride. An alternative method involved treatment of the second fraction with picrolonic acid, whereby the vitamin separated as the insoluble picrolonate. From this compound could be formed the hydrochloride.

The pure vitamin hydrochloride consisted of rosettes of small bars, melting at 250°, according to Jansen and Donath. The compound was readily soluble in water and methyl alcohol, but less readily in ethyl alcohol. With gold chloride the substance yielded a double salt, the analysis of which gave figures corresponding to formula $C_6H_{10}ON_2$ for the free base. Since the vitamin fraction responded with a positive Pauly's diazo reaction, Jansen and Donath stated that it probably contained an amidazole or a pyrimidine ring. The yield by their method was 100 mg. vitamin hydrochloride from 3 kg. rice. What was probably the most important feature was the test for activity of the compound upon bandols (native rice birds). Jansen and Donath reported that 0.0003 mg. vitamin hydrochloride administered daily to a bandol was sufficient to maintain health for three weeks in birds which otherwise would have contracted polyneuritis.

The effectiveness of Jansen and Donath's vitamin hydrochloride in both curing and preventing polyneuritis was attested by Eijkman (1927). He stated that a 0.5 mg. dose of the compound brought rapid recovery in chickens, and a 0.2 mg. dose produced the same beneficial result in pigeons. Further, the addition of as little as 4 mg. vitamin hydrochloride to a kilogram

of polished rice ration prevented the appearance of polyneuritis in pigeons. Eijkman reported that the active crystals gave a positive test with the Folin-Macallum uric acid reagent.

In contrast to these evidences supporting the isolation of the vitamin, it must be mentioned that Kinnersley and Peters (1927) applied the Jansen and Donath method to some yeast fractions without success. Furthermore, the substance producing the Pauly reaction did not fractionate with the substance possessing anti-neuritic activity. Kinnersley and Peters hesitated to cast doubt upon Jansen and Donath's work; rather the English investigators explained the failure of the method in their hands by assumption that the yeast used by them might have some peculiarity not possessed by rice polishings used by Jansen and Donath. It was also suggested that torulin might possibly be distinct from the anti-beri-beri vitamin.

It is unprofitable at present to discuss the conflicting statements in the above researches.

It should be remembered that when the studies reported in this chapter were made there was little or no evidence that what was called vitamin B consisted of more than a single nutrient principle. In 1926 Smith and Hendrick demonstrated that at least two principles, both indispensable, are contained in brewer's yeast and in extracts which had hitherto been used as sources of vitamin B. One of these, which is that now designated vitamin B, is destroyed by heating in an autoclave at 15 lbs. pressure for 2.5 hours, whereas the other is stable under these conditions. The oat kernel is rather poor in the latter substance, which is now designated vitamin G (the pellagra-preventive substance of Goldberger). A diet containing rolled oats as the sole source of both these vitamins is inadequate for promoting growth at the maximum rate, and is effectively supplemented by the addition of autoclaved yeast. The autoclaved yeast when fed with a mixture of purified food substances as the sole source of water-soluble vitamins is inadequate because the anti-neuritic vitamin B principle is destroyed. Such a diet becomes complete, however, when in addition to autoclaved yeast the active picrate of vitamin B prepared by Seidell is included. It will be appreciated, therefore, that we are unable at present to state in how far each of the components of the water-soluble vitamins played a rôle in certain of the experimental results described in this and the preceding chapters.

CHAPTER XV

EFFECTS OF VITAMIN B STARVATION ON METABOLISM

Several workers have reported investigations on the nature of the lesions produced when vitamin B is absent from the diet; but most of these are now of questionable value, since the preparations hitherto used to supply vitamin B provided at least both vitamins B and G, and so it is not clear whether the effects of deprivation for such vitamins were referable to one or the other deficiency. In some cases the diets were faulty in ways other than lack of water-soluble vitamins; and where pigeons were employed it is almost certain starvation played a major part in some of the results. This chapter should be read with an understanding that due caution is necessary in making any deductions from the experiments here recorded.

Carbohydrate Consumption and Beri-beri.—Funk (1914) observed that beri-beri did not appear in fasting birds and asserted that an increase in the amount of food ingested, especially of carbohydrate, hastened its onset. Vedder (1918) found that the rapidity with which polyneuritis develops in pigeons bears no relation to the amount of rice eaten but depends upon idiosyncrasy of the birds. His work demonstrated that the antineuritic vitamin (vitamin B) is not concerned with carbohydrate metabolism.

Green (1918) says the vitamin B requirement of pigeons varies with the exogenous metabolism and that vitamin consumption is no more related to carbohydrate than to protein or fat metabolism.

Changes in Blood in Avitaminosis.—Funk and Schönborn (1914) and Hirai (1921) found that in pigeons a lack of vitamin B resulted in an increased sugar content in the blood, and a diminished amount of glycogen in the liver. Abderhalden (1921) reported that pigeons fed polished rice have a red cell count of less than 50 per cent. normal.

Collazo and Bosch (1923) found that dogs fed a vitamin-free

diet showed a slight hypolipemia during the first 5 weeks of the experiment. This was followed by a stage of hyper-lipemia which was at its maximum at 11 to 13 weeks. Thereafter blood fat decreased markedly, almost reaching the normal value at the end of the sixteenth week, when death occurred. Alpern (1923) stated that the amino-acid nitrogen which ran parallel with the rest-nitrogen increased in both groups. The alcohol-ether soluble fraction also showed a slight increase. Collazo (1923) reported the carbon dioxide combining power of the plasma to be lowered in the last stages of avitaminosis in dogs, guinea pigs and pigeons.

Thompson and Carr (1923) found a marked increase in the uric acid and creatinin content of the blood with the onset of polyneuritis. Mattill (1923) stated that deprivation of vitamin B in contrast to fasting was not accompanied by an accumulation of nitrogenous products in the blood. Hayashi (1923) reports that the bicarbonate concentration of the plasma in beri-beri, excluding the cardiac form, varied from 0.692 to 0.13 per cent. in 14 cases. The normal values were 0.708 to 0.535 per cent. The urea and nitrogen content of blood was slightly higher than normal.

Effect of Starvation on Development of Polyneuritis.—Eijkman and van Hoogenhuyze (1916) pointed out that starvation contributes to the development of polyneuritis in birds as well as deprivation of vitamin.

Tissue Respiration in Polyneuritis.—Hess (1921) asserted that the pathological picture of pigeon beri-beri can be produced by means of hydrocyanic acid which inhibits tissue respiration. Gulick (1922) stated that the deficient calorie intake must be chiefly responsible for the lowered metabolism noted on a beri-beri diet. Abderhalden (1922) reported that symptoms of disease following the ingestion of a vitamin-free diet are always preceded by a fall of temperature of the body. He concluded that vitamins are in some way related to the oxidative processes.

Respiratory Quotient in Beri-beri.—Mattill (1923), from the data on the respiratory quotient of rats on rations with and without vitamin B, concludes that it is not related to the metabolism of carbohydrates, since after feeding cane sugar or glucose the quotients rose in both cases, slightly less rapidly in the vitamin-deprived group, due probably to delayed absorption.

Caridroit (1922) found that when pigeons are fed polished rice the respiratory metabolism remains fairly constant for the first week and then drops greatly. The respiratory quotient is at first

0.9 in both controls and tests, falls to 0.7 and is then increased to 0.8 at the moment of seizure. There is a marked decrease in nitrogen elimination. Magne and Simonnet (1922) reported that pigeons on a diet deficient in vitamin B showed a lowering of the respiratory quotient characteristic of starved animals. Parenteral administration of glucose showed that there was defective tissue oxidation.

Fleming (1923) made a metabolism study of patients suffering from beri-beri. The basic metabolic rate of these patients was the same as in normal controls. The respiratory quotients were likewise normal while the proportion of heat obtained from the oxidation of the various food-stuffs indicated a normal utilization of the food ingested. There was no evidence that a high carbohydrate diet was a cause of the disturbance. Beri-beri cases showed normal values for blood sugar, uric acid, urea and non-protein nitrogen, and this he interpreted to mean that there was no damage to the excretory power of the kidney in beri-beri even when edema was present.

Hofmeister (1923) regards vitamin B as an electronegative compound, since it can be adsorbed only in acid solutions. It is freed from the adsorbent only by alkali.

Catalase Content of the Tissues in Polyneuritis.—Studies made by Burge (1916-17) of the catalase content of the tissues of several species of animals (goose, pigeon, sparrow) indicate that it may vary in individuals of the same species and of similar weights and in a normal nutritive condition by as much as 100 per cent.

Dutcher (1918) was of the opinion that the carbohydrate effect was due to an overloading of the oxidative mechanism of the body rather than to a specific relation between the metabolism of carbohydrate and vitamin, the latter being used up in the process. He stated (1920) that the anti-neuritic substance functions as a metabolic stimulant, since the body temperature fell during the development of avian polyneuritis and rose after giving vitamin B. The catalase content of the tissues of polyneuritic birds decreased to 56 per cent. of the normal but returned to normal when they were given vitamin B. Such a depression of oxidation results, he believed, in the accumulation of toxic metabolic products, which affected the nervous system.

Stehle (1919) considers the variation in the catalase content of the blood and tissues to be satisfactorily explained as being due to variation in the number of red cells present. He regards

the glyoxalase content to be of no significance as an index to biological oxidation. Since Burge found the catalase content of normal animals to vary as much as 100 per cent., it is questionable whether these observations on polyneuritic pigeons have any real value. Findlay (1921) studied the effect of depriving pigeons of vitamin B on the content of glyoxalase in their tissues. Glyoxalase is an enzyme which plays a rôle in the metabolism of carbohydrate. It transforms "glyoxals" into lactic acid. The "glyoxal" which serves in the animal body as the intermediate substance between glucose and lactic acid is pyruvic aldehyde. Findlay found the amount of glyoxalase in the livers was reduced about one-half in polyneuritis. On inducing a "cure" by administering vitamin B the glyoxalase rose to two-thirds of the normal amount. Damianovich (1921) also reported that a diet deficient in vitamin B leads to a reduction of the amount of catalase in the liver.

Nature of Vitamin Action.—Walshe (1918) states that there are two factors in the production of beri-beri: the absence of a vitamin, and the use of certain foods which are the direct and immediate cause of the disease. He suggests that in the absence of a specific vitamin, carbohydrates undergo an aberrant hydrolysis with the production of toxic by- and end-products, thus producing beri-beri. Starved fowls live sufficiently long to develop beri-beri, yet they die without developing it, although they have been without the necessary vitamin.

Seidell (1921) compared vitamins as regards occurrence, stability to heat and alkali, solubility, dialyzability, loss of activity in fractionating procedures and amounts necessary for producing effects, and concluded that there is no reason why they should not be classed with the enzymes.

Wright (1921) believed that lack of vitamin B affects the functions of the intestinal canal, causing intestinal stasis and absorption of toxic products, and that these latter in turn resulted in diminished food consumption, loss of weight and ultimate death.

Burgi (1921) stated that the influence of large doses of morphine on the respiration of rabbits can be suppressed by administration of vitamin B without influencing the narcotic effect. The advantage of certain extracts of drugs over the pure substances may, he suggests, be brought into relation to the presence of vitamins in such extracts. He suggested that vitamins act as specific stimulants to the parasympathetic nerves and to the respiratory centers. Von Blohn, Santesson and v. Euler (1921)

regard the vitamins as substances related to the group of coenzymes or enzyme activators. Bickel (1922) asserts that vitamins do not influence either the digestion or absorption of food, but in the absence of vitamins the body cells gradually lose their ability to resynthesize and store digestion products. Gross (1924) also states that absence of vitamin B will produce intestinal stasis in the rat.

Are Vitamin B and Secretin Identical?—Vogetlin and Myers (1919) called attention to the fact that vitamin B reacted to chemical reagents such as methyl alcohol, silver, lead and barium salts in a similar way to secretin. Secretin preparations from the duodenum of hogs relieved to some extent the neuritic symptoms, and the vitamin B from brewer's yeast on injection into dogs stimulated the pancreatic and biliary secretions. A yeast preparation which had lost its curative powers for avian polyneuritis was devoid of stimulating effect on the pancreas and bile flow. Anrep and Drummond (1921) found that a yeast extract does not cause secretion of pancreatic juice as does secretin. Secretin can be extracted from the intestine of a cat showing polyneuritis. Cowgill and Mendel (1921) stated that extracts of yeast, rice polishings, wheat embryo, navy beans, and tomato, although containing vitamin B, were without noticeable effect on the rate of flow of pancreatic juice, bile or saliva in the dog. The intestinal mucosa of polyneuritic dogs was shown to contain secretin. The hypothesis that vitamin B stimulates these glands is not supported. Cowgill (1922) gave dogs a diet lacking in B. When this factor was given by a stomach tube there was a prompt recovery of appetite, which lasted from 4 to 19 days. Commercial extract of beef had no such effect, whereas a neutral aqueous protein-free vitamin extract, when given intravenously, promptly restored the appetite and was followed by a complete cessation of the nervous symptoms. He says (1923): "While the motility of the stomach is affected after a time by vitamin B deficiency, this relationship is not definite enough to warrant the conclusion that disappearance of hunger contractions is the immediate cause of appetite failure, the lack of vitamin B serving as the remote cause."

Histamine and Vitamin B.—Boyenval (1922) and Koskowski (1922) thought histamine may exercise an anti-neuritic effect. Histamine cannot replace the anti-neuritic vitamin.

Vitamin B in Relation to Appetite.—Karr (1920) studied the effect of specific fasting for vitamin B upon the appetite of dogs.

He fed isolated food substances essentially free from vitamins A, B and C. Casein and wheat gluten were the sources of protein. The dogs were restricted to these diets until they refused to take the food, and were then fed separately either yeast, milk, or tomato or a concentrated extract of vitamin B. These additions resulted in prompt response of the appetite, indicating that there was some relation between the desire for food and the amount of vitamin B ingested. Brewery yeast was much more effective than baker's yeast. He also studied (1920) the assimilation of nitrogen by dogs deprived of vitamin B. No decrease in digestion, nor in the character of the metabolic products eliminated could be detected. Vitamin A had no such effect in influencing the appetite as did B. Cowgill (1921) confirms Karr's view that "There is some relationship in dogs between the desire to partake of food and the amount of so-called water-soluble vitamin ingested." Alcoholic extracts of wheat embryo, rice polishings and navy beans improved the appetite.

Kennedy and Dutcher (1922) believe the effect of vitamins is not necessarily one of appetite stimulation but rather a stimulation of metabolic processes which promote growth or normal functioning. This appears to be a more logical view.

Beri-beri and Vitamin B Starvation.—Clair (1920) believes the condition produced in pigeons by feeding polished rice to have no connection with beri-beri. He believes the disease in man is due to toxic products of the bacterial decomposition of rice. Oseki (1921) asserts that the essential difference between human and avian beri-beri is that the former is due to an insufficiency and the latter to deprivation of vitamin B.

Hofmeister (1922) compared the anatomical findings and pathological symptoms of rats fed deficient diets; his studies indicate that in rat beri-beri there is no evidence of degeneration of peripheral nerves; hence this cannot be designated as true polyneuritis. In all severe cases there were alterations in the central nervous system such as hemorrhage in the cerebellum and medulla and consequent degeneration of the neural elements; the spread and number of hemorrhages were related to the intensity of the nervous systems. The pathological, anatomical diagnosis, he says, is not polyneuritis but cerebral purpura similar to hemorrhagic encephalitis which appears in chronic poisoning with alcohol, lead, and arsenic preparations.

Shinza (1923) reported the production in rabbits of a condition closely resembling experimental beri-beri when the animals were

given excessive amounts of sodium salts; he attributes this to the withdrawal of potassium from the body. Animals suffering from beri-beri could be cured, he states, by administering a potassium salt, whereas a sodium salt aggravated their condition.

Ogato and co-workers (1923) state that the syndrome developing from a deprivation of vitamin B and that of beri-beri are different. In polished rice disease (human beri-beri) the nervous symptoms develop toward the end of the course of the disease, while in experimental beri-beri they develop earlier. The pathogenesis of beri-beri lies, they believe, not in deprivation of vitamin B but in the toxin production by intestinal bacteria. Okada and co-workers (1923) state that in 7 out of 10 cases of vitamin B starvation the basal metabolism was decreased but became normal on administration of vitamin B. In beri-beri the basal metabolism was normal except in a few cases which showed marked lowering. Beri-beri is not, they believe, due to vitamin B starvation.

Changes in Internal Organs in Avitaminosis.—Abderhalden (1921) found that pigeons fed 2 weeks on polished rice had marked alimentary dystrophy. Simonnet (1921) showed that pigeons dead as a result of a deficiency of vitamin B usually showed no marked changes in the weight of liver, though in some cases there was distinct relative enlargement. The pancreas showed a marked reduction, connected perhaps with decreased amylolytic activity; the adrenals showed no certain weight changes; the thyroid in all cases was pale and deficient in volume; the thymus was absent.

Tiger and Simonnet (1921) stated that the pancreas of polyneuritic pigeons showed a slight reduction in diastatic activity insufficient to account for the symptoms induced by the diet, and probably secondary, developing from chronic avitaminosis. Findlay (1921) contrasted the livers and brains of normal birds with those in beri-beri: the latter showed a decrease in nucleic acid, most marked in the liver. Administration of vitamin B to a bird suffering from polyneuritis is followed by a removal of lipoids from the adrenal cortex, and an increase in the nucleic acid in the brain, the reappearance of the Nissl granules, and the disappearance of the paralytic symptoms. He concluded that vitamin B is an essential element in the synthesis of nucleic acids by the body. Rothlin (1922) found the content of diastase in the pancreas of beri-beri pigeons to be the same as that of normal pigeons.

Abderhalden (1922) observed that pigeons fed polished rice and treated with either the tissues of normally fed pigeons or with the tissues of polyneuritic birds reacted in the same manner. The alimentary dystrophy is, he says, the same in birds fed rice as in those fed purified food-stuffs without vitamins. Artom (1923) found that the cells of the pancreas of polyneuritic pigeons appeared full of zymogen granules and that the ducts were filled with stagnating secretion. During starvation the zymogen was reduced and the ducts contained no secretion. Korenchevsky (1923) states that the most characteristic changes in the glands of internal secretion in pigeons suffering from beri-beri are: hypertrophy of the suprarenals; and atrophy of the thymus and spleen, with persistence of the islets of the germ centers often accompanied by an increase in their number and size and frequent hypertrophy and hyperplasia of the islet cells. Adachi (1923) followed the uric acid and allantoin excretion in 3 dogs until death occurred on the forty-sixth, fiftieth and one hundred and third day of avitaminosis respectively. In the last animal the uric acid increased markedly as avitaminosis developed; the increase in allantoin excretion was not so great. In the other two experiments the results were not so clear. He concludes that "The increase in uric acid excretion depends on a disturbance in the metabolism of endogenous purines."

Rohr (1923) found that tissues may be arranged in the order of decreasing intensity of respiratory activity (measured by the capacity to reduce dinitrobenzene), as follows: kidney, liver, brain, and muscle. The hypothesis that in beri-beri there is a reduced oxidative capacity is thus supported. Hotta (1923) reports that the cholesterol content of nearly all body tissues increases during beri-beri—only the kidneys and testes appear to be exceptions. Noteworthy changes were not observed in the total phosphorus content. Asada (1923) found the body fat in rats suffering from avitaminosis to be less than normal. The diminution in cholesterol was not so great and the per cent. of cholesterol in total fat therefore increases. The results are taken to indicate an increased transport of body and food fats to the seat of fat combustion in avitaminosis. Pappenheimer and Larimore (1923) found that lesions occurred in the esophageal portion of the stomach in rats fed faulty diets. These consisted of an inflammatory edema of the mucosa and submucosa with marked cellular reaction and in most cases with localized ulceration, associated with the inflammatory changes. There was

always present more or less epithelial hyperplasia. These lesions were situated near the elevated ridge which divided the cardiac sac from the glandular portion of the stomach.

Miyadera (1921) found that animals had practically no gastric secretion when put on a vitamin-free diet; the diet was given until there was a marked disturbance. When 100 c.c. of a 5 per cent. solution of 96 per cent. alcohol was given a secretion was produced as great as before avitaminosis. He concludes that, in dogs, in avitaminosis the gastric secretory capacity is undisturbed, since in the vitamin-free diet there was a complete lack of stimulants for secretion.

Nerve Lesions and Absorption of H-ions.—Kato, Shizume and Maki (1921) stated that the nerve lesion resulting in paralysis in birds from feeding polished rice is due to absorption of H-ions. Kato, Shizume and Kitasato (1921) observed that the propagation of the nerve impulse decreases greatly in nerves of fowls suffering with polyneuritis, and that finally there is a total loss of propagation of stimuli. This is attributed to H-ions, since the same effect could be brought about by H-ions when allowed to act upon a normal nerve. Recovery from paralysis upon injection of rice bran extract is so rapid that the disease cannot be considered due to histological nerve degeneration. They believe it is caused by a poisonous substance. The sciatic nerve of fowls suffering from the disease had pH values 5 to 10 times greater than normal. The pH of the peripheral nerves was greater than the spinal cord. The pH of the blood of normal and diseased fowls was the same, but the power to neutralize acids was lessened in diseased fowls. Microscopic examinations showed the same degenerative changes in the myelin sheath in the axis cylinder in nerves paralyzed by H-ions as in the polished rice disease.

Asher and Kobori (1926) studied the effect of addition or subtraction of certain ions essential in the diet on the formation of internal secretions and of glycogen. On a calcium-poor diet the production of thyroxin was lowest, while on a calcium-rich food it was greatest. Adrenalin was formed in smallest amount on a potassium-poor diet and most abundantly on a potassium-rich food. On a calcium-rich food the adrenalin production was somewhat less than normal.

Changes in Adrenals in Polyneuritis.—Beznak (1923) asserts that in polyneuritic pigeons the adrenals increased 2 to 3 times in size, although the body weight and weight of the pancreas,

thyroid, liver and stomach, decreased. The hypertrophy was absent in starved animals and in those receiving only vitamin B. Thus the hypertrophy is not due to hunger. The per cent. of adrenalin in the adrenals was identical in both groups. In avitaminosis it is decreased. Gross (1923), contrary to the observations of McCarrison (1919), finds that absolute vitamin deficiency sets up very little alteration in adrenalin equilibrium, i.e., relation between adrenalin store, gland weight and body weight in the adult rat, provided the rat is not brought to the point of death on the diet. The alterations which do occur can be explained, he says, on a basis of general tissue atrophy. He believes that the striking changes reported by others have probably been produced by the accompanying starvation and unbalanced dietary rather than the deficiency in vitamins. It may be that the pigeon, monkey and guinea pig are predisposed to alterations to a much greater degree than is the adult rat. Stammers (1926) states that malnutrition in rats caused by deficiency of vitamins alone does not produce hypertrophy of the adrenals, and that there is little or no diminution in the lipoid content of the glands. More work must be done on this problem before definite conclusions can be drawn.

Infection and Vitamin B Deficiency.—Guerrini (1921) observed that pigeons whose vitality was lowered by restricting them to polished rice are no longer resistant to B. anthracis. The diarrhea of such pigeons is connected with a more strongly developed intestinal flora in which B. coli communis predominates.

Ishido (1922) reports that wound healing is delayed in guinea pigs and rats fed a faulty diet, e.g., a cereal grain, and the susceptibility to infection is increased.

Cramer (1920) described the "Lipoid gland tissue" found in the rat (1) at the back of the shoulders between the scapulæ, (2) in the thorax around the aorta, (3) in the abdomen between the kidneys and the abdominal aorta and surrounding the adrenal glands. In rats dead as a result of vitamin-free diets both fats and lipoids may be completely lacking in this tissue. It is suggested that this "Lipoid gland tissue" forms an important deposit of one or more of the vitamins. Cramer, Drew and Mottram (1921) report that the only demonstrable histological change in rats and mice in the final stage of avitaminosis is an extreme reduction of lymphocytes both in the lymphoid structures and in the blood. This condition of the lymphoid tissue is

practically identical with that produced by exposure to X-rays and radium. These agents not only have the same effect on the animals—they lose weight, and die in a state of emaciation —but also there occur in the seminiferous tubules degenerative changes similar to those observed in avitaminosis.

Changes in Reproductive Organs due to Lack of Vitamin B. —Novaro (1920) observed that pigeons fed a diet lacking in vitamin B exhibited degeneration of the tubules and hyperplasia of interstitial tissue in the testes. Dutcher and Wilkins (1921) found the testes of cockerels did not develop when fed polished rice. The addition of small amounts of fresh alfalfa caused the atrophied testes to increase in weight. In some cases the testes atrophied while the birds were slowly increasing in weight.

Evans and Bishop (1922), in their studies on the existence of a vitamin concerned with reproduction, comment on the disturbance of oestrus when vitamin B is omitted from the diet. Rats lost weight rapidly and in all cases cessation of oestrus occurred at once. When vitamin B was supplied they gained weight rapidly but did not have cycles for some time. Oestrus is not prolonged as in vitamin A deficiency, but is obliterated and the ovarian follicular apparatus comes to rest. They found ovulation to be more sensitive to a lack of vitamin B than general bodily nutrition.

Meyerstein (1922) states that the sex organs especially suffer in vitamin deficiency. The cells are poorly developed and fail in maturation. The interstitial tissue is relatively increased. Gotta (1923) fed pigeons a diet free from vitamin B and also with the addition of yeast. The testes and ovaries of the latter group were several times larger than those in the birds fed the vitamin-free diet whose gonads were atrophied. Souba (1923) found that in cockerels fed a diet deficient in vitamin B there was a decrease in the size and weight of the organs in the following order: testes, spleen, heart, liver, kidney, pancreas, and thyroid. Beaudette (1924) says that when hens were fed a ration poor in vitamins the number of fertilized eggs failing to hatch was larger than when the diet of the birds was normal and complete.

Evans (1928) has made a detailed study of the effect of inadequate vitamin B upon the sexual physiology of the male. He says that in all cases so far studied the diets have been low in E as well as in B and concludes that both acute and chronic deficiency in the anti-neuritic vitamin—deficiency leading to

death—leaves the anatomical and functional integrity of the male germ cell unaffected. It definitely decreases sex interest, but this effect may not be shown until a few days preceding death. Evans concludes that if vitamin E is adequately high, injury to the reproductive function in the male caused by inadequate B would appear to be due solely to this interference with behavior and to no extent to actual injury of the male germinal elements.

Refection.—Fridericia and co-workers (1927) describe a remarkable phenomenon under the term refection, in which experimental rats become immune to an exogenous source of vitamin B. A few rats which had been fed a mixture devoid of B grew thereafter and reproduced. All symptoms of B deficiency disappeared, the feces having become white and bulky. When young rats kept on a diet devoid of B are fed the white feces from refected rats for a few days, the refection is transmitted to them and may last for months or years. The feces and contents of the cecum contain the virus of refection. The virus does not pass the pores of Berkefeld or Chamberlain filters. The white feces from refected rats were kept in a dry state for 5½ months without losing the power to refect. The virus is not killed by heating to 80° for 10 months but was killed by heating at 100° for 10 months. The organs from refected animals were macroscopically normal. The spleen was normal in size in contrast to the atrophied spleen in rats fed a diet devoid of vitamin B. The digestive tract contained white material, half fluid, in stomach and ileum, coherent in the cecum and forming white feces in the colon. The contents of the stomach were distinctly acid. All organs examined histologically appeared healthy. No parasites, fungi or bacteria were observed.

The feces of refected rats differ from the gray or whitish feces occurring in man in sprue, and when the biliary duct is blocked. In these cases much fat and soaps are present or the biliary pigments are absent, but in refected rats neither of these abnormalities were present. The white color of the feces is due to a large content of starch.

The diet on which refection was observed consisted of purified casein 20 per cent., pure rice starch 57 per cent., butter fat 15 per cent., agar-agar 3 per cent., and salt mixture (185) 5 per cent. The feces of refected rats contained more amylase than brown feces from non-refected rats. This amylase was able to dissolve grains of rice starch. Neither the amylase of the white feces nor the ptyalin of human saliva is able to act to any

appreciable degree upon the starch contained in these white feces. The resistance of the starch to amylase appears to be due to some protective substance adsorbed upon the starch grains in these feces. This substance is soluble in alcohol.

Refected rats remain normal for more than 2 generations notwithstanding the absence of B from their food. Attempts to cause refection in adult rats devoid of B were unsuccessful. The white feces contained considerable amounts of vitamin B. The bacterial flora in the cecum of refected rats is different from that of other rats on the same diet. The cecal content of refected rats is acid as is that of normal rats, whereas the cecal content of non-refected rats on a vitamin B free diet is alkaline.

When refected rats are fed an adequate diet containing B in the form of yeast the defect in starch digestion disappears and the feces become normal. Attempts to cause refection in rats receiving an adequate diet by feeding white feces from refected rats do not influence starch digestion in the former. When refected rats growing normally are fed upon a diet devoid of starch as well as vitamin B their body weight decreases rapidly, but in spite of this decrease they live for a considerable time. Rats may be refected by a diet containing principally dextrin instead of starch. Roscoe (1927) and Kon and Watchhorn (1928) have confirmed these observations.

Since animals were in general deprived of both vitamins B and G in the experiments reported in this chapter, it is impossible to decide which factor was responsible for the effects observed. Since several observers have come to believe that there are three or more water-soluble vitamins, it is impossible until better controlled experiments are conducted to interpret satisfactorily experiments of the type described. The recent work of Palmer and Kennedy suggest that possibly other factors must also be considered. They (1927) have discussed the fundamental food requirements for growth in the rat. Young rats grow subnormally or not at all when fed a basal diet of specially purified casein, dextrin, wheat embryo extract, butter fat, mineral salts and agar-agar in proportions presumably adequate to satisfy the growth requirements. The animals were so housed that coprophagistic habits were prevented. They concluded that the requirements for normal growth involve other nutrients than those usually recognized and incorporated in their basal diet. The purification of the protein is especially important, and the basic deficiency or deficiencies can be brought about and aug-

mented by the biological changes in the digestive tract caused by the suppression of coprophagy. The dietary deficiencies manifested under these conditions appear to be of the nature of vitamin deficiencies. Vitamin A—carrying food substances or their extracts show a variation in their growth-promoting qualities not explained by the vitamins at present generally recognized.

CHAPTER XVI

BIOS AND THE GROWTH OF YEAST

The amounts of vitamins in any food are apparently very small and their separation laborious and costly. It is desirable, therefore, to estimate them by tests which are more accurate and also more economical of time and material than either the rat or pigeon tests. Before it became known that the water extract of yeast, wheat germ and other foods contains more than one indispensable substance the idea occurred to Williams that yeast cells might be advantageously employed as test organisms for estimating the anti-neuritic principle.

Yeast as Test Organism for Vitamin B.—Williams (1919) proposed such a test for vitamin B. He used as a culture medium a solution of inorganic salts and cane sugar, but the growth of yeast was slow and unsatisfactory. The addition of unknown substances which can be extracted from natural foods by water and dilute alcohol greatly stimulated the proliferation of the yeast. In hanging drop cultures the number of progeny of a single yeast cell, counted after an interval of a few hours, constituted the basis of judgment as to the vitamin content of the solution. This test appeared to be a great step in advance since it promised to detect very small amounts of the vitamin in a short space of time.

Bachmann (1919) found that all of the yeasts she used grew better and fermented more readily in a medium containing material extractable from natural foods. She also found that some yeasts would grow in and ferment a solution of inorganic salts and sugar even when introduced in small amounts. Bachmann states that Pasteur (1871) had already recorded this observation. She isolated a strain of yeast from canned pears which would neither grow in nor ferment a solution of inorganic salts and sugar unless large seedings were introduced, and even then fermentation was feeble. For this yeast some organic substance not contained in Nägeli's solution had to be added to stimulate budding. When substances especially rich in vitamin

B were added to the solution fermentation resulted. She stated: "My work suggests that such yeasts as this one might be used to determine the presence of certain vitamins and possibly also that they might be used to determine quantitatively the amount of vitamins present in various food substances."

Williams (1920) extended his investigations to the development of a gravimetric method, in which larger amounts of yeast were grown than in the hanging drop method. About 0.3 mg. of yeast was used for seeding and the harvest was collected after 18 hours on a Gooch crucible and weighed. He (1921) found that baker's yeast tested by the yeast growth method showed a much higher vitamin content than brewer's yeast, whereas by animal experiments brewer's yeast was the richer.

Emmett and Stockholm (1920) discussed the relation of the anti-neuritic and water-soluble B vitamin to the yeast growth-promoting stimulus. The rate of growth of yeast was accelerated by addition to the synthetic media of small amounts of preparations which they had shown by biological tests to contain the anti-neuritic and water-soluble vitamin (vitamin B). They concluded that the yeast-growth-promoting factor does not appear to be the same as the anti-neuritic or anti-beri-beri vitamin (by pigeon test). They believed the yeast growth stimulus to be different from the water-soluble, growth-promoting vitamin (by rat test).

Yeast Growth Not Satisfactory Test for Vitamin B Studies. —Souza and McCollum (1920) attempted to use yeast cell multiplication as a test for vitamin B and showed that no confidence could be placed in it, since yeast grows slowly in a vitamin-free medium. They used a solution of inorganic salts in which nitrogen and energy were supplied by ammonium sulphate and cane sugar respectively. Yeast grows slowly in this medium and the addition of extracts of natural foods which had been so treated as to destroy vitamin B were as effective in stimulating multiplication as were other extracts containing the vitamin. It is possible that the factor which is now known as vitamin G was responsible for the stimulation.

Funk and Dubin (1920) developed a quantitative method of measuring the yeast by collecting the harvest in capillary tubes by means of a centrifuge. Swoboda (1920) modified the Williams test and tested various extracts of animal organs. Miller (1920), using Swoboda's yeast method, studied the vitamin B content of vegetables. Whipple (1920) found the substance in

onions and cabbage which accelerates the growth of yeast is destroyed neither by heating to 100° for 30 or 60 minutes, nor by heating with acid (5 c.c. of vinegar to 100 c.c. of medium), nor with alkali (1.1 per cent. of sodium bicarbonate). Since vitamin G is so stable, possibly this factor was responsible for the results observed.

Fulmer, Nelson and Sherwood (1921) discussed several mediums for the growth of yeast. They state that the optimum concentration of ammonium chloride for the growth of yeast is that concentration of the salt in which a protein (wheat gluten) is least swollen. The optimum concentration of ammonium chloride varies with the temperature. At the temperatures studied the concentration of the salt causing the least swelling of the protein is identical with those most favorable for the growth of yeast. Treatment with alkali does not impair extracts of wheat germ or of alfalfa as yeast-growth stimulants. Evidently the stimulant is not vitamin B. A medium of known constituents was developed which promoted the multiplication of yeast without the addition of vitamins. Addition of vitamin B did not improve this. They concluded therefore that vitamins are not needed for yeast growth. Williams' medium gave a count of 130 when nothing was added; wheat germ extract raised the yeast count to 188; alfalfa extract raised it to 286, but neither could raise the yeast count when medium F was used. They also drew the conclusion that the yeast test has no specificity.

MacDonald and McCollum (1921) carried out an extensive series of transplantations of pure cultures of yeast in a nutrient medium containing no trace of vitamin B. They showed that a steady growth through many successive seedings is possible in the absence of the substance for which it had been suggested the growth of yeast could be made a specific test. The only objection which it was possible to raise against these experiments was the possible contamination of cane sugar of the purity of commercial domino sugar. Although they used samples of sugar purified by repeated precipitations from aqueous solutions by the addition of alcohol, the experiments were criticized by Funk and Dubin on the assumption that precipitation did not lead to purification. Fulmer, Nelson and White (1923) have answered this question with finality by employing in place of cane sugar a product, methose, prepared from formaldehyde. Methose is fermentable and utilizable by yeast. This product could be sub-

stituted for cane sugar without impairing the development of yeast in the synthetic medium.

Yeast Synthesizes Vitamin B.—Nelson, Fulmer and Cessna (1921-1922) demonstrated that yeast can synthesize vitamin B when grown in their nutrient media. This was shown by employing such yeast as the sole source of vitamin B in growth experiments with rats. MacDonald (1922) fed 5 varieties of yeast grown in a synthetic medium free from vitamin B and confirmed the findings of Nelson, Fulmer and Cessna that yeast can synthesize it.

Various Extracts on Yeast Growth and Activity.—Von Euler and Pettersson (1921) stated that the rate of galactose and sucrose fermentation by yeast was accelerated by lemon juice and wheat extracts even when the number of cells did not increase appreciably. Their experiments may mean that the formation of zymase is favored by vitamin B. Fränkel and Hager (1921-22) extracted animal organs with 80 per cent. alcohol. The extracts tested on yeast stimulated carbon dioxide production with the exception of bone marrow extract. Similar studies were made with plant extracts. In general, hull and root preparations were weakly stimulating, while leaf mixtures were active in advancing carbon dioxide production by yeast. The most active preparations were from lecks. Roasted coffee was an active stimulant, but green coffee was not; caffein gave negative results; choline inhibited, as did cholamine. Fullers' earth adsorbed the active principle to a great extent; kaolin adsorbed it completely, but aluminum hydroxid did not. Active preparations from yeast did not give a Molisch reaction. They considered the stimulating substance to be basic in character.

Dietary Properties of Yeast.—Funk pointed out that yeast is a rich source of vitamin B, and this has been repeatedly confirmed by others. Kennedy and Palmer (1922) found that yeasts from different sources vary in their content of vitamin B, and believe that it should not be used as a standard by which to compare other substances in feeding experiments.

Levene and Mulford (1923) observed that certain samples of yeast which would be a suitable source of vitamin B for growth of rats did not protect pigeons from polyneuritis when fed with polished rice. Five-tenths to two grams a day were given to different birds. They interpreted their results to indicate that there is a substance which promotes growth and another which prevents beri-beri.

Wildiers' Bios.—Wildiers (1901) observed that when a synthetic medium containing only well-defined substances was inoculated with yeast no growth occurred unless there was a large seeding. He explained the phenomenon by saying that the yeast required some unknown substance for its proliferation which the synthetic medium did not contain but which was present in beer wort; yeast cells also contained it. He termed this substance "bios" and attempted to study its chemical and physical properties. He concluded that yeast itself does not produce bios, and hence was dependent upon the nutrient medium for its supply. Pringsheim (1906) studied the phenomenon and concluded that the failure of small inoculations to grow in solutions containing only mineral salts and sugar was due to the inability of the yeast to adapt itself rapidly enough to its new surroundings; when a larger seeding was used some cells budded at the expense of nutrients derived from the dissolution of others. After repeated inoculations in the synthetic medium, single cells of wine yeast were observed to develop into colonies. He regarded Wildiers' bios simply as a suitable source of nitrogen for use of the yeast while adapting itself to the synthetic medium.

Wright (1922) described experiments consistent with this explanation: when small amounts of yeast extract were added to the synthetic medium the yeast grew at the expense of the nutrient principles supplied by the extracts until the concentration of cells reached a certain magnitude, after which budding could go on through the utilization of the nitrogen of ammonium salts.

Question of Identity of Vitamin B and Bios.—Lecoq (1920) asserted that the bios of Wildiers was not identical with vitamin B, but was probably the same as the auximones described by Bottomley (1914) which stimulate plant growth. Funk and Dubin (1921) separated from an extract containing vitamin B a substance which they called vitamin D, and which they believed to be necessary for the growth of certain microorganisms. Their vitamin D will be understood as the equivalent of bios.

MacDonald and McCollum (1921) described experiments in which yeast was grown in a purified nutrient solution of mineral salts and cane sugar of high purity, sufficient to furnish from 2 to 5 gm. of dry yeast cells. Successive seedings from a few cells into the synthetic medium were carried so far that a culture was secured which contained less than one five-trillionth of the bios in one loop of yeast cells used in the first seeding. Assum-

ing that the cane sugar was free from bios and from the anti-neuritic factor, it appeared that yeast may grow without either of these substances or it must be able to synthesize its own supply.

Ide (1921) called attention to the fact that there are two kinds of proliferation of yeasts: one very slow without bios and one fast with it. Without bios slow proliferation occurs but after several weeks weighable quantities of yeast were obtained. This had been observed by others. Ide suggested that the proliferation of yeast in the experiments of MacDonald and McCollum was due to impure cultures, but the latter state that "The successive seeding was continued to the fifteenth seeding with no apparent change in the rate of growth or diminution in the fermentative activity of the yeast. Every precaution was taken to insure pure seedings, a microscopic examination of a sample from each flask being made before seeding the next flask because of the known influence of other fungi."

MacDonald (1923) stated that extracts of yeast grown in distilled water plus pure cane sugar and nutrient salts served to stimulate the growth of seedings in such a medium in a manner comparable in its effectiveness with that of extracts of commercial yeast. Apparently yeast synthesizes a substance which stimulates the growth of the culture: this is the bios of Wildiers. It does not act as a vitamin, for an animal cannot grow without the necessary vitamins, whereas yeast can grow without bios. She further states that small seedings of yeast cells in the new environment of a fresh inoculation in a synthetic medium grow very slowly but proliferate much more rapidly when the special nutrient principle is supplied. Bios apparently does not act like a vitamin, since an animal cannot survive unless the vitamin is present. Although yeast can dispense with bios, it grows much more rapidly in its presence; it can synthesize it, but does so slowly.

Williman and Olson (1923) report that vitamin B is readily soluble in 95 per cent. alcohol, whereas bios is not. They list 60 nitrogen compounds which have been investigated, including 19 which they tested themselves; none of these were bios.

Fractionation and Isolation of Bios.—Devloo (1906), Ide (1907) and Frankel and Schwartz (1920) describe the fractionation of various extracts with a view to the isolation of bios. They appear to have assumed that bios is a single substance. Miller (1924) presented evidence indicating the multiple nature

of bios. Fulmer, Duecker and Nelson (1924) are in agreement with this view. They have separated a growth-stimulating preparation into 2 fractions, neither of which alone produces maximum stimulation, but do so when added together.

Eddy, Kerr and Williams (1924) have isolated from yeast a crystalline substance melting sharply at 223° C. which they believe to be bios. The recrystallized product retains its melting point and shows no loss of activity. The yield of the product was about 0.03 per cent. of the dry yeast. It contains 43.29 per cent. of carbon and 83.1 per cent. of hydrogen. The nitrogen content has not been accurately determined but is about 25 per cent. The substance gives neither the ninhydrin nor biuret reactions. It is easily soluble in cold water, in acid and alkaline solution, as well as in dilute ethyl alcohol. In cold 95 per cent. alcohol it is sparingly soluble but passes readily into solution when the alcohol is heated. It is only slightly soluble in 100 per cent. acetone but very slight dilution with water brings about its solution. The essential feature of the method of isolation consists of a preliminary purification of autolyzed yeast with fullers' earth which removes substances other than bios. Ferric hydroxid used in colloidal solution under specific conditions removes the bios which later can be dissolved and the bios freed from contaminating ions.

Levene and van der Hoeven (1924) prepared adenine hexoside from yeast. Its composition is C 44.41, H 5.09, N 23.57 per cent. and its melting point 214°. Analytical data, except for the hydrogen, remind one of those given by Eddy and co-workers. The adenine hexoside was tested by Levene for yeast growth but was not active.

Deas (1924) using malt combings which consist largely of sprouts of malt, prepared extracts from which two substances called bios I and and bios II were isolated. Bios I may be precipitated with barium hydroxid but is not adsorbed on charcoal, nor is it precipitated by lead acetate except in the presence of ammonia. It is not removed by shaking with yeast. Bios II is absorbed by charcoal, is removed from solution by shaking with yeast and is soluble in acetone. Excess of either of these two substances, especially bios II, is detrimental to the growth of yeast. Rats fed a diet deficient only in vitamin B were given 1 gm. daily of the rootlets in the form of a powder. Others were given bios I, others bios II, and others bios I and II in the same doses. Some rats were given an infusion of the rootlets. The

results showed that neither the rootlets nor the infusion contained vitamin B. Bios I and bios II, or both in combination so balanced as to promote abundant growth in yeast, were also inefficient in promoting the growth of rats. The substances do not cure beri-beri in pigeons. Deas draws the conclusion that Wildiers' bios and Funk's vitamin D are not identical with the rat growth-promoting vitamin B.

Unidentified Nutrients Essential for Micro-organisms.—Pacini and Russell (1918), working with rats, concluded that the typhoid bacillus produces vitamin B, but their experimental work is not convincing. Damon (1921) demonstrated that B. paratyphosus beta, B. coli, and B. subtilis do not produce vitamin B synthetically. Leichtentritt (1921) stated that malt, lemon and carrot extracts exert a stimulating effect on the growth of bacteria, especially staphylococci and diphtheria bacilli. This effect is not exerted by blood or ascitic fluid. György (1921) reported that "accessory substance" from milk and vegetables has the property of increasing cell respiration in isolated cells.

Thjötta and Avery (1921) found that the growth of B. influenzæ depends on two distinct and separate substances which are present in the blood. These substances occur in plant as well as in animal tissues. Sterile raw potato will serve as a substitute for blood in the cultivation of this bacillus. Broadhurst (1921) reports that approximately 20 species of bacteria on agar-agar cultures from 2 to 24 weeks old were revived by a sterile vitamin B extract of navy bean. Walker (1922) found that some of the more highly parasitic bacteria, e.g., the gonococcus, refuse to grow or grow only with difficulty on the ordinary culture media. An alcohol-ether extract of egg yolk or of fluids in which bacteria have grown and autolyzed for some time makes possible the growth of such bacteria. It is suggested that while most bacteria can produce for themselves any vitamins which they require, certain parasitic forms have lost this power. Kuroya and Hosoya (1923) state that polyneuritic pigeons are cured by feeding them B. coli grown on a synthetic medium.

Damon (1923) gives a review of the literature on the presence of growth-promoting substances in bacteria. He objects to the term vitamin as applied to any growth-stimulating substance of bacterial origin until such substance has been subjected to the biological test with a mammal. He found that 5 per cent. of a spore-forming organism, B. adhoerens, and 5 per cent. of a mucoid organism, Friedlander's bacillus, did not influence growth

in young rats. Five per cent. of Pfeiffer's bacillus, another mucoid organism, added to a diet deficient in vitamin B, maintained animals at a constant weight or induced rapid growth. Five per cent. of B. timothy, an acid-fast organism, had the same effect as Pfeiffer's bacillus. Ten per cent. of B. timothy induced rapid and continuous growth in rats.

Scheunert and Schieblich (1923) fed pigeons a vitamin-free diet until there was a marked decrease in weight and temperature. They then fed B. vulgatus which had been grown on a vitamin-free bouillon. Marked increase in body weight and rise in temperature followed. Hosoya and Kûroya (1923) studied the effect on bacterial growth of a vitamin B preparation prepared as a silver compound from the histidine fraction of the phosphotungstic acid precipitate of an alcoholic extract of bran. They describe a protein-free nutrient solution in which a considerable number of bacteria fail to grow appreciably, but are stimulated to growth by the addition to the medium of very small amounts of the vitamin preparation. They state that they are the first to obtain a protein-free synthetic medium suitable for the growth of meningococci. Shiba (1923) reported that the presence of vitamins A, B and C greatly favors the growth of B. typhosus, staphylococci and B. anthracis. Wyon (1923) believes that vitamin B is not essential to bacteria.

Drew (1923) found that substances necessary for continuous growth which are present in extracts of embryonic tissues are able to pass a Berkefeld filter. Adult tissues and tumors appear to contain these substances in lower concentration. Carrel (1924) in his studies of *in vitro* growth of tissues, emphasizes the importance of unidentified substances in the nutrient medium. He says that leucocytes, according to their condition, set free substances of varied nature, and that "trephones" are secreted by normal cells or diffuse from dead cells before the onset of disintegration.

Damon (1924) states that certain members of the acid-fat groups of bacteria produce substances which promote growth in rats: for example, an effect analogous to supplying vitamin B was obtained when B. smegmatis, B. timothy or B. moelleri constituted 7½ per cent. of the diet. Slanetz (1924) states that the following bacteria produce neither A nor B as determined by feeding experiments with rats: Azotobacter chroococcum; B. lactic acidi; B. mycoides; B. prodigiosus; B. radicicola; B. subtilis and Micrococcus agilis.

Observations on Vitamin Requirements of Plants.—Only a few studies have been made to determine the influence of unidentified nutrient principles on plant growth. Livingston (1907), when experimenting on the effect on plant growth of extracts of green and stable manures, discovered that organic material may be of direct importance. He added these substances to a slightly modified Knop's solution and found that "by far the most important factor in the effectiveness was the organic matter," which he found to be of direct value to the plant. Bottomley (1914) found green plants (lemon minor) in water culture grew better when certain organic substances were added. These were essential if the plant was to grow healthily and normally for any length of time. He called these substances auximones. They apparently function similarly to vitamins. They are produced during the bacteriological decomposition of peat by aerobic organisms, and are water-soluble. Mockeridge (1920) confirms the work of Livingston and Bottomley.

It will be clear that the nutritive requirements of micro-organisms have no similarity to those of mammals. It is possible that a series of unidentified substances may be found to determine whether certain species of bacteria or fungi can thrive in any medium. There have been no outstanding observations or advances during the past 4 years in the study of the vitamin requirements of micro-organisms.

CHAPTER XVII

THE DIETARY DEFICIENCY DISEASES—VITAMIN C

Scurvy

THERE has long been a belief among both medical and non-medical observers that the continued use of a faulty diet resulted in certain diseased conditions; of these, scurvy was the one most definitely attributed to unsatisfactory food. This disease occurred in the thirteenth century among the armies of the Crusaders. After the discovery of America, long voyages became more common and scurvy began to take a heavy toll of life among mariners. It became known as "the calamity of sailors" because of its frequency on shipboard. A notable instance was the voyage of Jacques Cartier in 1536. While in the St. Lawrence River he lost twenty-six of his party from scurvy but saved the remainder by the use of an infusion of pine needles (Hirsch, 1885). In 1747 James Lind, a surgeon in the British navy, studied the histories of sailors suffering from scurvy. By appropriate control experiments he showed that the medical treatment in vogue was futile, if not actually harmful, and that either orange or lemon juices was a specific cure. He studied the anti-scorbutic value of various fruits and green vegetables, but was unable to determine that any one was superior to another. Lind confirmed the observations of Kramer, made at the beginning of the eighteenth century during the war between the Turks and the Holy Roman Empire, that dried vegetables would not prevent scurvy. A friend of Kramer asserted "that no moisture whatever could restore the natural juices of the plant lost through evaporation," because these were "altered by a fermentation which they underwent in drying." Lind observed the beneficial effects of cow's milk in the treatment of scurvy. He explained this observation on the supposition of milk "being a truly vegetable liquor, an emulsion prepared from the most succulent wholesome herbs." Lind recommended that lemon juice concentrated to a syrup by evaporation should be carried on all ships

and served to the sailors. The efficacy of fresh fruits and vegetables as remedial agents in the prevention and cure of scurvy was discovered by common experience; and this had become so well established that in 1795 the British Navy began the regular administration of lime juice to the sailors. In 1865 the British Board of Trade adopted with equally beneficial results a similar regulation. Scurvy continued to be a menace to armies, appearing even during the World War because of a lack of knowledge among army officers of the dietary properties of foods.

Stevenson (1920) has given a very interesting report of an outbreak of scurvy in north Russia, together with an experiment in test dieting during the World War. It appears when there is absolute deprivation of fresh food that scurvy may appear after about 6 weeks. The presence in the food of even very small amounts of articles which provide vitamin C will defer the onset of the disease so that it may be months before it develops.

Professor Hopkins (quoted by Dupain, 1920) described the experience of a large preparatory school in England. During a winter term the conduct of the boys grew "vaguely unsatisfactory." The standard of work and play fell much below normal, the boys became listless and irritable and various forms of minor complaints were reported. The explanations forthcoming were insufficient. Throats were examined, drains flushed, and other hygienic measures were instituted, but nothing came to light, and the conditions grew worse. It was suggested that the diet be inspected. It was found that the diet supplied nothing in the way of uncooked foods. The shop where the boys had purchased fruit with their pocket money was closed. Upon a liberal introduction of fresh fruit and raw vegetables into the dietary the whole trouble disappeared. The school had been suffering from incipient scurvy, due to lack of vitamin C.

Symptoms of Scurvy.—Lind, in discussing scurvy in sailors, made an observation which deserves our careful consideration to-day in relation to the effect which the diet may exercise on general well-being and behavior. He says: "An uncommon degree of sloth and laziness which constantly accompanies this evil (scurvy) is often mistaken for the wilful effect of the patient's disposition. This may prove fatal to many, some of whom when obliged by their officers to climb up the shrouds have been seen to expire and fall down from the top of the mast."

Scurvy develops gradually. The adult loses weight, is anemic, pale, weak, and short of breath. The gums become swollen,

bleed easily and frequently ulcerate. The teeth loosen and may drop out. Narcotic areas in the jawbone may result. Hemorrhages into the mucous membrane and the skin are characteristic. In the skin large blue-black spots develop after trivial injury, or even spontaneously. The ankles become edematous, and in severe cases there develops a hard, boardlike condition of the skin and subcutaneous tissues. Nervous symptoms of various types are seen. In the later stages of the disease, headache, convulsions and delirium appear.

Lime Juice Not Always Protective Against Scurvy.—Although the experience of the British Navy and of several Arctic expeditions had convinced many persons that lime juice was a specific remedy for scurvy, there were a number of widely known outbreaks of the disease which tended to discredit the view that scurvy was due to the lack of some essential substance in the diet. One of these was the experience of Sir George Nares, who in 1875-77 made an expedition to the polar regions in the *Alert* and the *Discovery*. Both crews suffered severely from scurvy, although lime juice was regularly rationed to all. It appears that the lime juice was inferior, and perhaps adulterated. Smith (1919) states that what we now call the lemon was frequently called lime. The true lime is very inferior to the lemon in its anti-scorbutic property.

In 1895-96 the British Army garrisoned at Chitral, India, suffered severely from scurvy, notwithstanding the fact that lime juice was administered daily. In 1902 the men who constituted the party of Captain Scott in his voyage to the Antarctic developed scurvy in spite of the fact that they were well supplied with West Indian lime juice. On this occasion the men were saved by the substitution of fresh seal meat for canned meats; mustard and cress were grown for consumption in the raw state. Probably the lime juice used in those cases where there was no protection against scurvy was adulterated, or had been heated to preserve it. Heating (contact with air) destroys the substance which gives fresh fruits and vegetables their protective value.

Theories Concerning Etiology of Scurvy.—The scurvy outbreaks, despite the use of lime juice, aroused in many minds the suspicion that scurvy might not be due to lack of freshness in the food. A number of theories were advanced to explain the etiology of the disease. Jackson and Harley (1900) accepted the view advanced by Torup of Christiania, that scurvy resulted

from eating decomposed meat, and was a kind of "ptomain poisoning." This view seemed plausible. When the disease was common the sailors were restricted essentially to a diet of salt meat and hard tack. The experiences of men in the Arctic regions with fresh and stale meats confirmed the supposition that the latter were inferior to the former. Stefánsson (1918) concluded that a factor of importance was the consumption of large amounts of salts with salted meats. He denied salt to his men as a protective measure against scurvy. It is doubtful whether the consumption of salt was in any way related to the incidence of scurvy.

Scurvy Attributed to Acidity of Diet.—Wright (1895) compared the symptoms of scurvy with those induced by large doses of mineral acids, and from their similarity concluded that scurvy was due to acid intoxication. He suggested that diets of cereals or of meat produced scurvy because they contain an excess of mineral elements which in metabolism are converted into acids. The following table gives a list of foods, with their relative yields of acid and base radicals, presented by Wright in support of his view. The values represent the oxalic acid equivalent of 100 grams.

TABLE XXI

Acid Food-stuffs	gm.	Alkaline Food-stuffs	gm.
Oats	1.69	Carrot	0.61
Barley	1.19	Turnip	0.32
Beef	0.27	Potato	0.27
Wheat	0.25	Onion	0.25
Egg	0.20	Cow's milk	0.17
Rice	0.10	Ox blood	0.13
Maize	0.07	Peas	0.13
		Lemon juice	0.12
Neutral Food-stuffs		Orange juice	0.12
Sugar		Beans	0.07
Fats		Sheep blood	0.07

The presence of organic acids in certain of the vegetable foods in the alkaline list is of no significance in this connection because these are destroyed by oxidation in the body.

Those foods which were reputed to prevent or cure scurvy fall in the alkaline group. Wright believed that it was not essential for the diet to contain uncooked succulent foods, but that it should supply sufficient alkaline radicals to make the diet somewhat basic.

First Observation of Scurvy in Guinea Pigs.—The first to observe scurvy in guinea pigs as the result of restriction to a faulty diet was Theobald Smith (1895). He limited his guinea pigs to a diet of oats and bran. This chance observation was not followed by a detailed study and remained unnoticed for more than twenty years.

Classic Experiments of Holst and Frölich.—Modern knowledge of the etiology of scurvy dates from the discovery by Holst and Frölich of the University of Christiania (1912) that guinea pigs readily develop the disease when confined to a diet of cereals or of bread. It was characterized by loosening of the teeth, inflammation of the gums, hemorrhages in the joints, decalcification of the epiphyses of the long bones, and the typical changes in the bone marrow. Exclusive feeding of guinea pigs with carrots, turnips or dandelion greens did not lead to this condition, although animals on these foods suffered comparable losses in weight (30 to 40 per cent.). The addition of small amounts of fresh cabbage or carrots or other fresh vegetables cured the animals suffering from scurvy. They concluded that scurvy was due to the absence of a certain chemical substance from the "scorbutic" diets.

Infection Theory.—The work of Holst and co-workers did not explain the etiology of scurvy to the satisfaction of all. Jackson and Moore (1916) observed that guinea pigs fed a diet of oats and milk ad libitum developed typical scurvy. Bolle (1902) was apparently the first to state that guinea pigs become scorbutic when fed exclusively on raw or cooked milk. Hart and Lessing (1913) produced scurvy in monkeys by feeding condensed milk, rice and dried pignuts. Milk is known to induce growth for a time in young animals during the suckling period when it constitutes the sole source of nutriment. This naturally suggested that some factor other than diet entered into the etiology of scurvy. Jackson and Moody (1916) isolated a diplococcus from the tissues of scorbutic guinea pigs which, when subsequently inoculated into pigs kept on a diet capable of maintaining health, produced hemorrhages; some weeks later the organism was again isolated from the lesion. These findings afforded evidence that scurvy may be induced by bacterial infection.

Faulty Deductions of McCollum and Pitz.—The observation that guinea pigs develop scurvy when fed on oats and milk seemed very significant to McCollum and Pitz (1917). As a result of studies on the rat, McCollum and Davis (1915) formu-

lated the working hypothesis that the simplest diet which can support normal nutrition in this species is one which fulfills the following conditions: (1) it must contain sufficient protein of the "complete" type; (2) it must furnish adequate amounts of nine mineral elements in appropriate combinations (Na, K, Ca, Mg, Cl, P, S, Fe, I); (3) it must supply a source of the carbohydrate glucose; (4) it must contain two dietary factors of undetermined chemical nature, which McCollum and Kennedy (1916) provisionally called fat-soluble A and water-soluble B.

McCollum had found that a diet of oats and milk sufficed to maintain a rat in health. When McCollum and Pitz, independently of Jackson and Moody, observed that guinea pigs confined to a diet of oats and milk quickly succumbed to scurvy, they found it difficult to believe that the disease could be due to the lack of a specific substance, for milk alone suffices as the sole food for all young mammals during a critical period of their lives.

Their guinea pigs suffering from scurvy developed impaction of the cecum when the condition was brought about by feeding milk and oats, both constipating foods. They therefore offered the tentative explanation that injury to the cecum of this species is of primary importance as a factor in the etiology of scurvy. They accepted the view of Jackson and Moody that the cause of the disease was an invasion of the tissues by bacteria, made possible by the damage to the cecum as a result of the retention of feces. Paraffin oil or cathartics sometimes saved scorbutic animals from death. In the light of later studies McCollum and Pitz accepted the view that scurvy is a deficiency disease.

Early View of Hess on Cause of Scurvy.—After the appearance of the paper of McCollum and Pitz, Hess (1917) presented a study of infantile scurvy which strongly supported the view that scurvy is not simply a deficiency disease. He reported that infants fed milk pasteurized at 145 degrees F. for thirty minutes did not develop scurvy, whereas others fed the same milk, which had been allowed to age for twenty-four hours on ice, did so. He stated that milk pasteurized at 165 degrees was more likely to induce scurvy than milk which had been pasteurized at the lower temperature, and that aging of raw milk increased its liability to cause scurvy. The degree of heat to which the milk had been subjected seemed not to be the most important factor, for according to his statement boiled milk did not produce the disease. The most plausible deduction from these observations appeared

to be that boiling tended to render the milk sterile, whereas heating to 165 degrees killed most of the lactic acid-forming bacteria, leaving the resistant spore-forming organisms in the milk. The latter could develop rapidly because with aging the lactic acid content would not rise and hold them in check, as is the case in milk soured in the normal manner. Hess concluded that it was the staleness and the bacteriological condition, rather than the temperature to which the milk had been heated, which determined whether or not it would cause scurvy in an infant. As the result of later studies Hess (1920) abandoned this theory and accepted the view that scurvy is a deficiency disease.

These papers stimulated the study on the etiology of scurvy, and the various theories concerning its etiology were tested by experiments. The papers of Chick and Hume, and Cohen and Mendel, deserve special consideration, since they explained the errors into which several workers had fallen, and established the correctness of Holst's view that scurvy is a deficiency disease.

Chick and Hume Confirm Views of Holst.—Chick and Hume (1917) extended the observations of Holst and his co-workers by making a comparative study of the values of a number of foodstuffs as protectives against scurvy. In the light of the data of Chick, Hume and Skelton (1918) the erroneous conclusions of McCollum and Pitz were explained. Chick and co-workers showed that the anti-scorbutic value of milk had been overrated. They adopted hand feeding, instead of allowing the guinea pigs to eat milk ad libitum, and showed that when less than 50 c.c. of raw milk daily supplemented a diet of oats and bran it did not defer death from scurvy beyond about 30 days. When 50 c.c. of raw milk were consumed daily the life of the animals was extended to about 75 days. An intake of 100 to 150 c.c. of milk daily served to maintain the guinea pigs free from scurvy for 16 weeks, when the observations were discontinued.

It is necessary, therefore, that the diet should consist almost entirely of milk if, through it alone, scurvy is to be prevented. This concept is consistent with the rôle played by mammalian milk, which is destined to form a complete food for a definite but limited period in the life of the young.

Studies of Cohen and Mendel on Scurvy.—Cohen and Mendel (1918) were the first to produce scurvy in guinea pigs on a diet which was complete from the nutritive standpoint except for vitamin C, and which was suitable in its physical properties for this species. Any animal which possesses so highly specialized

a digestive tract as the guinea pig, in which the stomach and cecum are very large and delicate, cannot be satisfactorily nourished on a diet which is thoroughly digested and absorbed, and which leaves little residue, or one which forms hard or pasty feces. The cereal grains leave little residue, and oats and milk form hard feces. Cohen and Mendel used a diet of cooked soy bean flour supplemented with 3 per cent. each of sodium chloride and calcium lactate, and dried brewer's yeast, and sufficient raw Jersey milk to furnish 5 per cent. of butter fat. The yeast was added to provide vitamin B, and butter fat to furnish A.

On this diet, complete except for vitamin C, young animals gain weight rapidly, but about the tenth day, while still eating well and gaining in weight, they begin to show tenderness of the wrists and ankles. Their condition after this time rapidly becomes worse, and the joints swell to two or three times their normal size. The appetite fails and there is a sharp decline in weight. At this point the animals can be saved by the administration of foods containing sufficient vitamin C.

With this diet Cohen and Mendel confirmed the observations of Holst and of Chick and co-workers. They describe what appears to be simple scurvy unaccompanied by other conditions due to malnutrition. They have given the most complete description of the syndrome of scurvy in the guinea pig. Its onset is characterized by tenderness of the wrists, ankles and knees, developing in the order named. Within a day or two the affected joints become swollen, frequently to the extent of two or three times the diameter of the normal bone. Spontaneous fracture of the bones is of frequent occurrence. These conditions are observed especially in guinea pigs which weigh 150 to 250 gm. Older animals lose the use of their hind legs because of stiffening and paralysis. The animals find relief in lying on the side or back so as to favor the affected members. This has been described by Delf (1918) as the "face-ache" position. If they are permitted to remain long on the diet deficient in vitamin C the swellings fail to disappear when the diet is corrected, and develop into exostoses. When curative measures are administered early enough the bones appear to return to normal.

Pathological Findings in Scurvy.—The two most noticeable conditions observable at autopsy are hemorrhage and fragility of the bones. The stomach, intestines and cecum may show congestion, hemorrhage or ulceration, but may in some cases appear

normal. These pathological conditions are usually observed in animals fed oats and water or oats and milk, and less frequently in the ones fed the diet of Cohen and Mendel. An ever present condition in advanced scurvy is loss of appetite. The food intake diminishes as the disease advances, but fasting does not produce the lesions of scurvy.

It is remarkable that the swollen joints and tenderness often appear while animals are still growing rapidly and have good appetites. Inanition can, therefore, play but a minor rôle, if any, in the production of the more prominent symptoms of scurvy.

LaMer and Campbell (1920) found an increase in the weight of the adrenal glands which may represent a compensatory response to the decreased adrenalin production known to exist in a scorbutic animal. The heart and kidneys, they believe, had increased in weight, but the livers of scorbutic animals did not appear to be enlarged.

Morel and co-workers (1921) state that the bone changes seen in experimental scurvy are not related to defects in calcium metabolism. Morikawa (1920) observed the following changes in the suprarenals in experimental scurvy in guinea pigs: increase in weight, increase in lipoidal content of the cortex, and reduction in amount of doubly refracting fat. He states that the lipoid in the zona fasiculata was distributed in 3 layers, viz., an outer layer rich in lipoids, a middle layer poor in lipoids, and an inner layer rich in lipoids. McCarrison (1921) has also reported enlargement of the suprarenal glands in the guinea pig during experimental scurvy. The changes were slight during the first fifteen days, but during this period the weight of the spleen, liver, stomach, intestines and their integument were definitely reduced. These results have been confirmed by Bessesen (1922). Wells (1921) says the tooth pulp is abnormal in scorbutic guinea pigs. Hess, Unger and Pappenheimer (1922) found prolonged exposure to direct sunlight did not prevent scurvy in guinea pigs. Gerstenberger and Burhams (1922) found that scorbutic infants and guinea pigs, as well as polyneuritic pigeons, can burn carbohydrates completely.

Iwaduchi (1922) found a decrease in amount of ash in muscle and bone from scorbutic guinea pigs; the fat content of muscle and adrenal was decreased; the content of calcium and of phosphorus in the adrenals was below normal; the calcium content of the blood was 50 per cent. below normal; the protein content

of the blood was subnormal, and haemoglobin was markedly decreased, although the number of red corpuscles was essentially normal. Iwaduchi, however, could not confirm the observations of others that the calcium and phosphorus content of the bone was abnormal.

Liotta (1923) found a marked diminution in the number of red corpuscles, as well as in the amount of haemoglobin, and a slight increase in the number of leucocytes in the blood of guinea pigs in scurvy produced by a diet composed exclusively of oats. Höjer (1924) has discussed the pathology of the bones, teeth, muscles, heart, liver, spleen, kidney, adrenal and salivary glands, lung, blood, blood vessels and connective tissue in scurvy in the guinea pig.

Shipley, McCollum and Simmonds (1921) found the bones of rats in polyneuritis brought on by a diet adequate except for lack of vitamins B and G to be quite indistinguishable from those of guinea pigs dying of scurvy. The bones were very osteoporotic. One received the impression that all growth must have stopped. The epiphyseal cartilage was very shallow (only 4-5 cells in depth) and stained intensely with basic dyes. The marrow contained comparatively few nucleated cells (leucocytes and their predecessors). The blood vessels of the medullary cavity were crowded with red blood cells. In many places the vessels had ruptured and numerous small and large hemorrhages were to be found, especially in the vicinity of the cartilage. In some bones the active bone marrow had been almost completely replaced by hemorrhages. The bones of rats fed the same diet with 30 per cent. of rolled oats as the source of vitamins B and G revealed no lesions whatever, notwithstanding the specific fasting of these rats for vitamin C.

Harden and Zilva (1920) found that monkeys of 2 to 3 kilos body weight were protected from scurvy by the small amount of orange juice that was required to protect a guinea pig of 300 to 400 gm., viz., 2 to 5 c.c. per day. The monkey develops scurvy in about two months, whereas the guinea pig requires only about two weeks. Mouriquand and co-workers (1924) found that an attack of scurvy renders animals more susceptible to subsequent attacks. Anderson and Smith (1924) studied the effect of severe acute scurvy on the subsequent growth and food intake of guinea pigs. The loss of body weight in scorbutic guinea pigs at the height of scurvy cannot be accounted for by inanition alone. Growth in the post-scorbutic stage is either parallel to or greater

than that of the normal pig, and is accompanied by a definite increase of food intake.

Lindsay and Medes (1926) and Medes (1926) studied the histological changes in the testes in scorbutic guinea pigs. A diet deficient in vitamin C or one containing sufficient A, B and C, but inadequate as to amount, caused degeneration of seminal epithelium unaccompanied by any marked changes in the intertubular tissue. Pigs killed after ten days on a scurvy-producing diet showed degeneration of germinal epithelium, especially of the cells in the early stages of spermatogenesis, although there were present no clinical symptoms of scurvy. Guinea pigs cured of severe chronic scurvy showed almost complete recovery of germinal epithelium. The scurvy-producing diet consisted of alfalfa meal and wheat flour, equal parts by weight, oats ad libitum and powdered whole milk (Klim) corresponding to about 50 c.c. of fresh milk daily. It is interesting to note that the germinal epithelium showed degeneration, since this diet contains liberal amounts of vitamin E according to Evans and Bishop's early work.

Smith (1927) studied the alimentary tract in scurvy in the guinea pig. The diet was similar to that used by Medes, but did not contain milk powder. By the methods employed X-ray and dye were used in the study of motility; alimentary albuminuria, glycosuria, pentosuria in the study of permeability) no significant impairment of the gastro-intestinal tract of the guinea pig in acute or chronic scurvy were revealed.

Meyer and McCormick (1928) have recently published a valuable monograph on the pathology of scurvy.

Metabolic Disturbances in Scurvy.—Höjer (1925) observed in scorbutic tissues no marked diminution in the intensity of respiration as measured by Thunberg's methylene blue method. Palladin (1924) states that hyperglucemia develops in scorbutic guinea pigs. This is denied by Mouriquand and co-workers (1925). Mouriquand and co-workers (1924) also studied the changes in the iron content of the blood as scurvy progressed in guinea pigs. The normal iron content was 0.53 gm. per kg. of blood, and this value was found up to the twenty-fourth day as scurvy developed. After that the iron content diminished, ranging from 0.21 to 0.40 by the thirty-first day. When an anti-scorbutic diet was resumed the iron in the blood returned to normal in 5-7 days.

Mouriquand, Leulier and Michel (1925) state that analyses

of bones and teeth show that there is practically no alteration in their calcium or phosphorus content. They suggest that the fragility of scorbutic bones is due to changes in the ossein. They also state that lack of vitamin C exercises no influence upon the cholesterol content of the blood, but that of the suprarenals is greatly reduced.

Palladin (1924) states that during scurvy in guinea pigs creatine appears in the urine, the creatine content of the muscles is increased and the creatine coefficient is greater than normal. These increases parallel the severity of the disease and may be referred to disturbed carbohydrate metabolism. He reports a diminution in the excretion of ammonia. The catalase content of the blood increased and that of esterase diminished during the second week of the disease. Peroxidase, after an initial rise, returned to its normal level. Protease increased during the terminal stages of scurvy.

Palladin and Ssawron (1924) found that the calcium excretion in scorbutic guinea pigs decreased in most cases from 17 mg. per two-day excretion to 12.8 mg. In the third week it dropped to less than half the normal value. Death then resulted. The decrease of calcium in the feces was less marked, while that of 5 c.c. of blood decreased from 0.625 to 0.439 mg. Jarussoqa (1926) found both oxygen consumption and carbon dioxide output decreased in experimental scurvy, the latter to a much greater extent than the former. The provision of cabbage greatly stimulated energy metabolism.

Lepper and Zilva (1925) found large variations in the bicarbonate content of the plasma, whereas the pH remained nearly constant. The low titratable alkali observed in the blood of scorbutic guinea pigs could be restored to normal by the administration of citrates, without influence upon the onset of scurvy. Mouriquand and co-workers (1927) determined the pH of the blood of scorbutic guinea pigs, but failed to find any evidence of acidosis until after the twenty-seventh day, after which time the alkali reserve fell irregularly to about one-third by the fortieth day. They found the addition of sodium bicarbonate, citrate or lactate did not hinder the development of scurvy and that the lesions are not a result of acidosis. They found the adrenalin content of the adrenals normal in scurvy.

Wolbach and Howe (1926) suggest that scurvy is the result of an inability of the supporting tissues to produce and maintain intercellular substances. This results from the absence of an

agent common to all supporting tissues which is responsible for the setting or jellying of a liquid product. Vitamin C is liberated in the destruction of tissues. Osteoblasts, in spite of great change in morphology, preserve their chemical potentialities and produce bone matrix.

Randoin and Michaux (1926) found on the average 5.9 gm. of cholesterol per 100 gm. of fresh suprarenals in normal guinea pigs and 2.63-2.78 gm. per 100 gm. in scorbutic animals.

Certain Species Immune to Scurvy.—It has been impossible to produce experimental scurvy in the rat. One must conclude that the rat either does not require the anti-scorbutic substance in its metabolic processes, or has the capacity to produce the substance synthetically.

Parsons (1920) produced acute scurvy in guinea pigs. She then fed the fresh livers of rats reared on a diet free from vitamin C to the scorbutic guinea pigs and observed prompt cure. The rats were able to synthesize vitamin C. McCollum and Parsons (1920) fed a young prairie dog a diet which would produce severe scurvy in a guinea pig within 30 days. It grew in a nearly normal manner and showed no signs of scurvy at the end of ten months.

Parsons and Hutton (1924) and Lepkovsky and Nelson (1924) extended these studies on the synthesis of vitamin C by the rat. They carried rats into the second generation on a scurvy-producing diet, and cured scurvy in guinea pigs by feeding the rat livers. Bailey and Hyde (1923) have shown that hemagglutinins which exist normally in the blood of the adult fowl are not present at hatching, but appear at a definite time afterwards; those active for the rat and rabbit cells appear about the sixteenth day, whereas those active for guinea pig cells appear about 3 weeks later. It would be interesting to inquire whether vitamin C is present in livers of newborn rats, when it is present in the diet of the mother and when it is lacking.

Parsons and Reynolds (1924) found livers from normal guinea pigs cured scurvy in other pigs; livers from scorbutic pigs failed to cure the disease. Man, guinea pig, swine, monkey and dog are all susceptible to scurvy because they lack the synthetic power possessed by the rat.

Vitamin C Requirement of Rabbits, Calves and Dogs.—Findlay (1921) and Lopez-Lamba (1923) found that rabbits on a scorbutic diet can reproduce and induce normal growth in the young during the suckling period. Suckling rabbits are very

sensitive to a lack of vitamin C, and since they remain in health the milk of the nursing mother must provide this substance. The non-susceptibility of the rabbit to scurvy, Findlay concludes, must be looked upon as relative rather than absolute. When deprived of vitamin C they lose weight and eventually die. Thurston, Eccles and Palmer (1926) found that calves do not require vitamin C in quantities which can be measured by the present method of testing food materials for anti-scorbutic potency. It seems probable, therefore, that cattle, like the rat, are independent of vitamin C in the food supply because of an ability to synthesize this principle.

Lavialle (1927) demonstrated that dogs fed a diet containing vitamins A and B, but deficient in C, develop scurvy in 7-10 months. The dogs fed milk which had been concentrated, homogenized and sterilized did not develop scurvy in 15-16 months, but if milk was heated to 120-125° for 45 minutes and fed as the sole source of C, scurvy developed in 9-10 months.

Birds Do Not Require Vitamin C.—Plimmer and Rosedale (1923) kept pigeons during 15 months without a source of vitamin C; during this period the birds laid eggs and reared young. They kept chicks from one day after hatching to maturity without vitamin C; these birds laid eggs and a few young were hatched. Ducks, geese, turkeys, guinea fowl and pheasants were reared from the day-old stage to ages of 12 to 16 weeks without vitamin C. Carrick and Hauge (1925) found that fowls show no apparent ill effect after several months' deprivation of anti-scorbutic substance, and their livers still contain it, as shown by tests on guinea pigs.

Formation of Vitamin C in Germination.—Fürst (1912), working in Holst's laboratory, found that whereas dry cereals or pulses did not prevent scurvy, they acquired anti-scorbutic properties when allowed to germinate. He was the first to point out the possibility of producing the anti-scorbutic principle at will by the germination of seeds. Wiltshire (1918) and Chick and Delf (1919) confirmed Fürst's observations on the value of germinated seeds in the treatment of scurvy.

Honeywell and Steenbock (1924) find that vitamin C does not occur to an appreciable degree in barley soaked in water 24 hours, or on soaking for 96 hours in the absence of oxygen so that germination does not occur. Barley germinated in the absence of light contains C. Hart and co-workers (1925) concluded that the disappearance of vitamin C from fermented corn (silage)

and sauerkraut must be due to the oxygen retained in the mass.

Chemical Investigations of Vitamin C.—Hess and Unger (1918) showed that when orange juice is precipitated by the addition of 95 per cent. alcohol or absolute alcohol, the solution remains anti-scorbutic, but the precipitate is of no value. The vitamin is soluble in eighty per cent. alcohol. They (1918) also reported that orange juice after it had been boiled and made alkaline to litmus by adding sodium hydrate just before using can be given intravenously to scorbutic infants. According to Harden and Zilva (1918) orange juice made alkaline equivalent to N/20 with NaOH and stored 20 hours in a cold room did not delay the onset of scurvy in guinea pigs when doses of 5, 6, or 7 c.c. daily were given. Samples of juice alkalinized to N/50 on the date of feeding and then fed in doses of 3, 5, or 7 c.c. did not prevent scurvy. Alkalinity is very destructive of vitamin C.

Vitamin C from lemon juice from which citric acid had been removed failed to pass through a collodion membrane which had been soaked in 90 per cent. alcohol, but did pass through a membrane soaked in 95 per cent. alcohol (Zilva and Miura, 1921). Both vitamins B and C diffuse through a collodion membrane permeable to substances like methylene blue, neutral red and safranin.

Hess (1921) states that copper greatly accelerates the destruction of vitamin C, and that the small content of copper in milk which has been in contact with utensils containing it catalyzes its destruction. Two and one-half parts per million of copper added to milk appreciably reduced the anti-scorbutic value during pasteurization.

LaMer (1921) concludes that vitamin C is neither a protein nor an enzyme. The rate of inactivation by heat increases with a decrease in hydrogen-ion concentration. He believes the inactivation is due to intramolecular oxidation and reduction such as occurs in aldehydes and sugars.

Hart, Steenbock and Lepkovsky (1922) found vitamin C soluble in 80, 95 and 100 per cent. ethyl alcohol. It is soluble in methyl alcohol, but insoluble in butyl alcohol, benzene, petroleum ether, acetone, ether, chloroform and ethyl acetate.

Zilva (1922) found that decitrated lemon juice of pH 6.8 could be boiled for 2 hours in an atmosphere of carbon dioxide without destruction, whereas in a single hour in a current of air its potency was reduced to less than 25 per cent. Ozone destroys

vitamin C. It can also be destroyed by passing air through a solution containing it at ordinary temperatures. Two hours' boiling in the presence of carbon dioxide did not destroy it. One hour's boiling in air did destroy it. Hydrolysis of decitrated lemon juice with hydrochloric acid for 5 hours destroys some vitamin C present in the juice, but a considerable amount was still present.

Zilva (1924) concentrated decitrated lemon juice to one-tenth volume and acidified it by adding 7 gm. of citric acid to 1 liter of juice. This was introduced into flasks which were evacuated, sealed and stored in the laboratory in the dark. Samples were tested 3 and 5 months later and no loss of potency could be detected. Invert sugar in decitrated lemon juice may be fermented without appreciable alteration.

Bezssonov (1925) has obtained from fresh cabbage juice kept out of contact with air a substance crystallizing in needles from absolute alcohol or acetone, which in daily doses of less than 2 mgm. prevents scurvy in guinea pigs. It appears to be an O-diphenol. On oxidation it yields a quinone with phenolic qualities, and which appears to be a derivative of anthracene. He states (1926) that 1.5 c.c. citron juice daily was necessary to protect a guinea pig against scurvy; 0.75 c.c. fed in conjunction with 60 c.c. of cow's milk heated to 120° C. also afforded protection. On the basis of these and other studies (1927) he concluded that vitamin C consists of two substances, one of which, C′, is less resistant to heating in an autoclave than is the second component C″. The first, he suggests, corresponds to the substance separated by Scotti-Fogleine (1926) by distillation. The latter distilled a solution containing vitamin C, collecting fractions up to 100°, 120° and 134° C.; none of these prevented scurvy. The residue heated to 120° C. was completely effective. The distillates gave no color reactions for vitamin C, and the residues only faintly. Bezssonov believes that vitamin C has a volatile portion which aids in giving the characteristic color reactions.

Vedder and Lawson (1927) found that when lemon juice is evaporated to dryness after neutralizing with calcium carbonate, vitamin C was dissolved by ethyl alcohol, phenyl methyl alcohol, and in a mixture of 75 parts of ethyl acetate and 25 parts of alcohol. It also dissolved in acetone on the addition of a small amount of alcohol. Neutral lead acetate removed most of the

phosphorus and sulfur from such extracted materials without impairing their curative value.

Zilva (1925) states that lead acetate does not precipitate the anti-scorbutic principle but that basic lead acetate precipitates it quantitatively. The precipitation of the vitamin by lead acetate is most effective within a range of pH 5.4 to 7.2. He found no relation between the amide nitrogen content and anti-scorbutic value of his preparations.

Daubney and Zilva (1926), and Hoyle and Zilva (1927) found no nickel, cobalt, manganese or boron in concentrated preparations of vitamin C, but phosphorus, iron, sulfur and iodine were present. Zilva (1927) found that phenolindophenol is rapidly reduced in the air to leuco-base by decitrated lemon juice and by active vitamin C fractions from the same source. The reducing substances, although very closely associated with the vitamin, are not identical with it; he suggests that its presence may contribute to the stability of C in that juice.

Tests Proposed for Vitamin C.—Bezssonov (1921, 1922 and 1924) has described a phosphotungstic-phosphomolybdate reagent which does not give a reaction with monophenols, except a yellow brown with guaiacol, and no color with uric acid or glucose. With diphenols it gives a persistent intense blue with hydroquinone; with pyrocatechol and catechol a red violet turning to blue, but no color with orcinol and resorcinol; a yellow brown with protocatechuic acid, pyragallol and tannin. All anti-scorbutic extracts gave a blue color. After treatment which destroys the vitamin the blue color was diminished or not produced. He believes that the color is due to an unstable radical, probably one of the polyphenols which in solution is easily split off from the vitamin. Kay and Zilva (1923) found that the majority of anti-scorbutic liquids give the color reaction, but a preparation from lemon juice did not, although actively anti-scorbutic. Koopman (1924) found that comparative tests agree in general but vary with milk, the color reaction being negative, but the test with the guinea pig positive. Loewy (1925) states that the test of Bezssonov gave results in good agreement with clinical observations and experiments on animals, and apparently corresponds to the vitamin C content of different foods. Sajous (1924) has described a test for vitamin C. In this, the food is crushed, or rasped, and its fluid is treated with a few drops of 1-1,000 adrenalin chloride solution. A few drops of a

solution composed of one part of 3 per cent. hydrogen peroxide solution to nine parts of water is then added. When mixed in a test tube the two solutions give no change of color. It is only when a tissue or fluid contains the oxidizable vitamin that reaction occurs. A positive test is indicated by the development of a red color. Sajous refers to this as the "adrenin H_2O_2 test." Unfortunately no color reaction for any vitamin has been fully proven to be specific.

Vitamin C and Ultra-violet Rays.—Zilva (1919) studied the effect of ultra-violet light on vitamin C. Lemon juice brought to H-ion concentration of pH 2.34, and also some which was made neutral, was exposed to these rays for 8 hours without influencing its properties. Hess, Unger and Pappenheimer (1922) found that prolonged exposure to direct sunlight did not prevent scurvy in guinea pigs. Clark (1925) and others have shown that irradiation of guinea pigs fed a scurvy-producing diet is without effect in postponing the onset of the disease. Reyher and Meller (1926) found that irradiation of cow's milk with ultra-violet rays destroyed vitamin C. Ozone appeared to be the destructive agent. Eggleston and Harris (1925) stated that direct exposure of an animal on a scorbutic diet to ultra-violet light did not retard the onset of scurvy and had no curative effect on the scorbutic animal. Irradiation of cooked cabbage did not confer anti-scorbutic properties upon it.

Anti-scorbutic Properties of Food-stuffs.—Hess (1917) found yeast to be without anti-scorbutic value. He found wheat germ to possess anti-scorbutic value but less than orange juice. He states that infants may develop scurvy while gaining weight.

Hess and Unger (1918) obtained good results in the treatment of scurvy in guinea pigs with canned tomatoes. They recommended 30 gm. of canned tomatoes per day for infants as an economic and efficient anti-scorbutic. Young fresh carrots withstood dehydration, retaining some curative properties. Delf (1918) estimated that 70 per cent. of the vitamin is destroyed by one hour's heating at 60°, and over 90 per cent. in the same time at 90°.

Lewis (1919) found that 25 gm. of banana was the minimum protective dose which protected a guinea pig against scurvy. Eddy (1926) and von Meysenbug (1928) have recently reported that the banana contains considerable vitamin C. Dutcher and co-workers (1919) found raw lean beef to possess no anti-scorbu-

tic properties. Chick, Hume and Skelton (1919) found that dried tamarinds, cocum and mango possessed definite but small anti-scorbutic value.

Givens and co-workers (1922) found that raw apples and bananas possess anti-scorbutic properties but that much of their potency is lost in cooking. They found 10 gm. of raw apple or 1.5 c.c. of lemon juice to be the minimum protective dose for a guinea pig. Eckman (1922) found dried peaches, apricots, apples, pears, prunes, cherries and loganberries do not prevent scurvy in guinea pigs when 4 gm. per day are fed. Peaches delay but do not prevent scurvy.

Embrey (1923) found that 10 gm. daily of pomelo, cucumber, chico and guava, protected guinea pigs from scurvy for 9 weeks, whereas 15 gm. of banana, kangkong leaves, and camote leaves were essential for similar protection. Höjer (1924) found 25 c.c. of commercial whortleberry juice failed to protect guinea pigs. Five c.c. of strawberry juice showed some protective effect. He found that 3 c.c. of the juice of tomatoes fully ripe, but produced late in the season, had very little protective effect on guinea pigs. Raspberry juice and black currant juice were of little or no value as anti-scorbutic foods.

According to Steidle (1924) mushrooms either fresh or dried possess no anti-scorbutic value. Delf (1925) found that vitamin C exists in stored fruits or roots as long as the tissue itself remains alive. She found about one-half the normal value of vitamin C in the juice of oranges and lemons stored 5 years in a frozen condition, whereas the juice of swedes lost most of its value under similar conditions in less than 2 years. Canned orange and tomato juices retain much of their vitamin C after 4 years' storage at laboratory temperature. Vitamin C is more stable in orange juice than in lemon or tomato juice. Vagliano (1925) found anti-scorbutic value in a product consisting of 4 parts of orange juice to 2 of sugar and 1 of water, heated 1-2 hours at 115-120°, after six months of preservation. Willimott and Wokes (1926) report that the flavedo of ripe lemons contains but small amounts of vitamin C. According to Merjanian (1926) both grapes and grape wine contain vitamin C, the amount varying with the kind of grapes and their freshness. Old wines contain but a trace, and dried grapes none. Medin (1926) found cloud-berries equal to lemons in anti-scorbutic value.

Miller (1925) found vitamin C in fresh pineapple. Hauge and Carrick (1926) found fresh egg white and egg yolk unable to

prevent scurvy in guinea pigs. Brouwer (1927) reports vitamin C in fresh grass.

Stability of Vitamin C.—Holst and Frölich (1912) found the anti-scorbutic potency of fruits and vegetables display greater stability in acid than in alkaline solution. Fruit juices were more heat stable than vegetable juices; they suggested that the acidity protected the anti-scorbutic principle.

Anderson, Dutcher and co-workers (1921) made the interesting observation that vitamin C was stable to heat (within limits) but was easily destroyed by oxidation. Milk which had been boiled without stirring contained vitamin C, but milk pasteurized and stirred had lost most of this factor.

Sherman and co-workers (1922) made an elaborate study of the anti-scorbutic value of tomato juice. They found that 2 c.c. of fresh tomato juice enabled guinea pigs to live 70 to 90 days, but at autopsy hemorrhages of a mild character were seen.

Vitamin C in Canned Foods.—Kohman and Eddy have studied the preservation of vitamin C in canned foods. Fruits peeled and cut, and immersed previous to canning in 2 per cent. salt solution until the oxygen in the juice is used up by respiration retain their vitamin C content. Cold storage apples lost considerably in C content but when the fresh fruit was canned by the method described no loss of C was noted after 8 months' storage. In making apple sauce and baked apple most of the C is destroyed.

Raw spinach they found exceptionally rich in A and C. One gram or less of the raw product daily contains enough C for the guinea pig; 0.026 gm. daily sufficed for vitamin A for the rat. The B content is less in spinach than A or C, and amounts to one-third to one-sixth that of yeast. Cooking raw spinach by the usual methods has little effect on A, but heating at 100° for 10 minutes in a kettle destroyed 95 per cent. of C. They found it possible to heat spinach after canning from 1-2 hours at 115° and still have several times as much C left as in the kettle-cooked article. The exclusion of oxygen is the most important factor in preserving vitamin C. Commercially canned peaches contained 4-5 times as much vitamin C as the kettle-cooked fruit.

Anti-scorbutic Value of Powdered and Heated Milks.—Hart, Steenbock and Smith (1919) found commercial unsweetened evaporated milk had lost its anti-scorbutic properties. Hart, Steenbock and Ellis (1921) reported that milk powders vary in their vitamin C content with the process of manufacture. The

spray process is more destructive of vitamin C than the Just process in which the milk is dried on a drum.

Anderson, Dutcher and co-workers (1921) reported that boiled milk was practically equivalent in anti-scorbutic properties to fresh unheated raw milk. Pasteurized milk heated at 63° C. for 30 minutes did not prevent scurvy. The pasteurized milk had been stirred vigorously while heating, whereas the boiled milk had remained undisturbed. The pellicle formed on the surface protected it against the oxygen of the air. Destruction of vitamin C is due to oxidation. Oxidizing agents such as hydrogen peroxide quickly destroy this factor.

Jephcott and Bacharach (1921) compared summer milk, winter milk and neutralized milk dried by a roller process with milk powder dried by the spray process. They found milk powders prepared from both summer and winter milks by the roller process to possess anti-scorbutic values about equal to the original raw milk from which they were prepared. The neutralized milk contained less vitamin C than raw milk and the spray processed dried milk was markedly deficient in anti-scorbutic value.

Clark and Collins (1922) concluded from their studies that milk powders and their remade products are safe for infant feeding. Since raw milk varies in its vitamin C content, milk powders must also vary in this factor, and it would seem the part of wisdom to give all infants and young children fed milk powders suitable anti-scorbutic food daily.

It appears that some of the experimental work on the anti-scorbutic value of milk powders has been carried out under the auspices of commercially-interested people; it is not always possible to determine what data should be given credence.

Kinugasa and Hattori (1924) compared (a) milk heated at 100° for exactly 30 minutes and quickly cooled, (b) milk heated at 70°, (c) that treated with the flash method of pasteurization and cooled in 20 minutes from incipient boiling, and (d) ordinary pasteurized milk from the Japanese market. Guinea pigs fed (a) died of scurvy on an average of 23 days, those fed (d) in 20 days, those fed (c) in 22½ days, and those fed (b) in 26 days. Smith (1924) found that human milk dried by the roller process retained about 80 per cent. of its anti-scorbutic value when freshly prepared, and about 40 per cent. after 2 years. Schmitt (1925) found fresh goat's milk effective as an anti-scorbutic, but buttermilk much less so.

Relation of Food of Cow to Vitamin C of Milk.—Barnes and Hume (1919), Dutcher, Eccles and co-workers (1920-21) and Hart, Steenbock and Ellis (1920), have studied this problem. The last named have reported studies of the anti-scorbutic value of milk of cows which had been kept on dry feed as contrasted with that of cows which were feeding upon green pasture. Summer milk was found to be much richer in vitamin C than winter milk. Fifteen cubic centimeters of summer milk afforded protection against scurvy to a guinea pig during twenty weeks, when superimposed upon a scorbutic diet. But for most individuals, however, as much as 50 c.c. daily were necessary to afford complete protection. Of winter milk from cows fed exclusively on dry feed for many months, 70 c.c. were necessary. Silage or sugar mangels added to a ration of dry feeds did not improve the anti-scorbutic value. Dutcher and his co-workers (1920-1921) found 20 c.c. of summer milk superior to 60 c.c. of winter milk in vitamin C. When cows were changed from a ration of fresh green feed, to one of dry grains, hay and fodder, the milk did not fall off rapidly in its anti-scorbutic value, but only after the lapse of some weeks did it become inadequate. When cows were changed to a green pasture after a long period on a dry feed, their milk very promptly improved in its value as an anti-scorbutic agent. Hess, Unger and Supplee (1920) confirmed the observation that milk of cows on dry feeds is deficient in vitamin C.

MacLeod (1927) found the milk from stall-fed cows in winter to have considerable anti-scorbutic value. Hughes and co-workers (1921) found that the milk of cows which had been fed during a year on foods containing very little of vitamins A, B and C, still contained approximately the normal anti-scorbutic value. Such milk contained a much lower content of fat-soluble vitamins than that from cows on a diet rich in these principles. Two cows fed vitamin-deficient diets for a year produced normal calves. They further confirmed these findings (1927) that the vitamin C content of cow's ration has little, if any, influence on the anti-scorbutic value of the milk. Synthesis of vitamin C by the lactating cow seems the only explanation for the results obtained.

Diet of Nursing Mother Should Contain Fresh Foods.—Cow's milk, and probably human milk, has relatively low anti-scorbutic value even when derived from a satisfactory diet. It protects the young when the diet is almost wholly milk. When

top milk is modified by dilution and sugar or cereal water added, the infant may be in danger of scurvy. Few infants have recognizable scurvy before the age of 9 to 10 months. The reason for this is not clear. It is not known whether the infant is born with a reserve of vitamin C, as with iron, or whether it possesses in early life the power to synthesize it.

Further evidence relating to the importance of the mother's diet to the anti-scorbutic value of her milk is afforded by a report of a committee of the American Pediatric Society in 1898. Among 356 cases of scurvy in infants 10 had been nursed. The following table suggests the anti-scorbutic potency of milks treated in different ways.

TABLE XXII

Relation Between the Kind of Food and the Incidence of Scurvy

Breast milk	12 cases; alone in 10
Raw cow's milk	5 cases; alone in 4
Pasteurized milk	20 cases; alone in 16
Condensed milk	60 cases; alone in 32
Sterilized milk	107 cases; alone in 68
Proprietary infant food	214 cases

These data show that a small amount of vitamin C remains in heated milk. The occurrence of a small number of cases among nursed infants shows that the meat, bread and potato type of diet so extensively used in this and other countries in recent times, does not suffice for the production by a nursing mother of milk which is complete for the nutrition of her infant. We have no way to determine how large a number of infants, as a result of taking breast milk of poor quality, have reached the stage of borderline cases of scurvy.

Infantile Scurvy a Recent Condition.—Infantile scurvy is not often mentioned in early medical writings. Not until the middle of the nineteenth century did it attract the attention of medical observers. Until the last few decades infants were either breast fed or they died. Not until the eighteenth century was artificial feeding or mixed feeding mentioned in medical literature. Bread and water, called "water pap"; bread and pulse; small beer with egg, etc., were the common foods administered to infants who had lost their mothers. Usually they died from intestinal infection or inanition before they had time to develop scurvy.

Underwood, in 1793, was the first to use cow's milk as a food for infants. About the middle of the nineteenth century the sale of proprietary foods became important; doubtless there was a

parallel increase in infantile scurvy. The development of bacteriology led to the discovery that typhoid fever, bovine tuberculosis, scarlet fever, septic sore throat and other diseases might be milk-borne, and health officers urged the pasteurization of the milk supplies of cities. While this protected adults against epidemics which had been shown to follow milk routes, it was disastrous to many infants. Pasteurized milk was deficient in vitamin C, and scurvy among infants increased as the artificial feeding came more into vogue. Only through the researches of the last 2 decades has this danger to infants become fully understood, and the means of correcting this deficiency discovered.

Barlow (1894) first described a diseased condition which we now know to be a complication of rickets with scurvy in infants. The syndrome became known by his name. He states that the disease was new in England, but more common in Germany, and that it appeared to be on the increase both in England and America. His paper is the earliest and best description of scurvy in infants.

It is interesting to correlate the history of human scurvy with the changed conditions under which large groups of persons have lived at different times. In early times the disease affected chiefly soldiers and prisoners, since they were subjected to simple and monotonous diets consisting of dry, cooked or stale foods. Dry foods keep well, and are selected by those who purvey for large masses of people, especially when transportation or storage is an important matter. With the advent of steam navigation no special precautions are necessary for the protection of seafaring men against this disease, since they frequently have access to fresh food.

Investigations as to the cause of scurvy give us another example of animal experimentation contributing a blessing upon the human race. Without systematic experiments with the guinea pig years or decades would certainly have passed before the cause of scurvy would have been understood. Thenceforward we may confidently look for the protection of babies, soldiers, explorers and prisoners, as well as the general population, against this malady. Commander Byrd in 1928 went to Antarctica provided with dry beans which might be germinated in case of necessity to provide his men with an anti-scorbutic food.

CHAPTER XVIII

ABNORMAL SKELETAL DEVELOPMENT IN CHILDREN

VITAMIN D

Rickets

Glisson's Studies on Rickets.—In 1650 Glisson published the first adequate description of the disease known as rickets. The condition had appeared in the counties of Dorset and Somerset in England about 30 years previous to his writing, and was called "rickets" by the country people from the Old English "wrikken," which means to bend or twist.

Barlow's Disease.—Barlow (1894) discussed the relationship of infantile scurvy and rickets and expressed his belief that these were true cases of scurvy, due to inadequacy of "living food" reacting on a child with a rickety basis. He described the disease as having a sudden onset at any period after four months, but occurring chiefly between the ninth and eighteenth months. The child was generally rickety but was fairly well clothed as far as subcutaneous fat was concerned. From its association with rickets it became known as scurvy-rickets. The symptoms are well marked, consisting of anemia, hemorrhage from the gums, extensive subperiosteal hemorrhages, and intense pain in the bones and joints, so that the child fears the slightest movement.

Barlow's disease and rickets are not necessarily diseases of the poor. The former occurs chiefly among children who are well cared for and whose state of nutrition is good so far as fat and weight are concerned. It seldom occurs in breast-fed children, whereas rickets not infrequently occurs in the latter. It arises chiefly in infants of the better class who either have been kept on some proprietary food, especially those mixed with water or with condensed milk, or who have been kept on condensed milk alone, or pasteurized or sterilized cow's milk.

Age Incidence.—Rickets appears in infants as early as the third month. Prematurely born children are especially susceptible to the disease. Dunham (1923) reported well-developed

rickets in a baby 34 days of age. The blood showed a normal calcium but a low inorganic phosphorus content. She believes that this is the earliest age at which rickets has ever been proved to be present. The disease usually manifests itself from the sixth to the twenty-fourth month. Although it is usually a disease of infancy, it sometimes attacks older children and even young adults and is then called late rickets. Children with rickets are usually fairly well developed and appear to be fairly well nourished.

Clinical Symptoms.—Rickets is a disease which affects the entire body, although the most noticeable signs of it are seen in the bones. At its beginning the children are usually constipated, restless and irritable, usually apathetic and disinclined to play. They sleep poorly. Frequently a rachitic child rolls its head on the pillow until the hair is worn off from the back of its head. The child perspires freely, especially about the head.

The muscles are lax and the tendons and ligaments may become elongated; because of this, and the softening of the bones, children do not walk or stand at the proper time. Although they have opportunity for exercise, they do not profit by it like the normal child. The muscles of the intestines and abdomen are so weakened that a "pot belly" develops. The muscles of the legs and arms are also weak.

As the disease progresses the deformities of the bones begin to show. One of the first to appear is the "rachitic rosary"—a line of knobs on the side of the chest where the bones of the ribs join the cartilages. The walls of the chest are drawn inward every time the child breathes, and grooves are formed along either side of the line of attachment of the diaphragm. The chest becomes compressed from side to side and "pigeon breast" deformities develop. Bosses of new bone are formed on the side and front of the skull, and the head acquires a square shape. The ends of the long bones of the extremities become enlarged. The legs become knock-kneed or bowed. The bones of the arms bend, and there is marked enlargement of the epiphyses at the wrists and ankles. In severe cases curvatures of all sorts appear. Some children show very severe anemia during the course of the disease and some manifest extreme nervousness and even convulsions.

Rickets by itself is seldom fatal, but the child may die from some complication. Park and Howland (1921) have stated that if a child with rickets does not die during the first year or so

from some complication, it may improve in health, even though the disease continues and lime salts are not deposited in the bones. The shafts of the ribs and the costo-chondral junctions may acquire ultimately a sufficient strength to withstand the forces exerted upon them. If given sufficient time, osteoid tissue will be produced in sufficient quantities both in the cancellous tissue under the periosteum and in the metaphysis itself to supply the requisite rigidity. Even though the costo-chondral junctions remain weak, respiration can take place in a normal or approximately normal manner, provided the shafts of the ribs acquire sufficient strength. Extreme deformities of the thorax are frequently seen in children 3 or 4 years of age. While rickets is often looked upon as a disease which in itself does not threaten life, they state that it does threaten life and may be the direct cause of death when it deprives the thorax of its rigidity. It increases the danger to life in respiratory infections, for example, tuberculosis and pneumonia.

Occurrence in Animals.—Hansemann (1906) stated that the successful feeding of carnivorous animals during infancy and adolescence was a very difficult matter. He pointed out that in the wild state while still very young they eat glandular organs and chew soft bones, and under these conditions do not develop skeletal abnormalities; but when kept in confinement and fed upon muscle meat and body fat, and given a bone so large and hard that little can be gnawed from it, they invariably develop rickets. The dog develops rickets, whereas the domestic cat does not: the cat kills birds, rodents and other small animals, and thus supplements its diet and escapes rickets; the dog adapts itself more readily to domestication and limits its food to that provided by its owner.

Cheadle and Poynton (1908), in discussing the conditions in the London Zoo, brought out several interesting points. They had had much difficulty in rearing lion cubs. Cubs were fed horse flesh and had invariably died with extreme rickets; more than twenty litters had been lost in this way. At the suggestion of Bland-Sutton, a litter was given pounded bones, cod liver oil and more milk, in addition to the raw meat. These cubs were kept in the same dens and under the same conditions of temperature, illumination and ventilation as before. The change in the condition of the animals was very remarkable; they grew to be strong and healthy.

In many large zoos there has been difficulty in rearing young

carnivora. In most of these places it is now the practice to supply them with liver, flat bones containing much marrow, and fat; and at intervals of a few days to give them either rabbits or small birds, such as pigeons, which are consumed entirely. Confinement does not seriously interfere with their successful development under these conditions of feeding. They should, however, be exposed to sunlight.

Jost and Koch (1914) stated that rickets is a very common disease among pigs, puppies, lambs and kids, but is less common among colts, calves, and rabbits.

Geographical Distribution.—Rickets is most prevalent in children in Great Britain, France, Belgium, Germany, European Russia, Austria, Hungary, and the United States of America. Countries in higher and lower latitudes are much less subject to the disease. In Greenland and the Faroe Islands, and in Iceland, it rarely occurs; it is very rare among the Eskimos and Lapps. Hirsch (1885) noted that Finsen, in 5 years' practice in Iceland, saw only 42 cases and these were of a mild type. In Norway and Denmark it has a subordinate place in the statistics of sickness relating to the earliest years of life. Very few cases are found in southern Italy and the southern provinces of Spain and Greece. Rickets is usually thought to be rare in Turkey, but recent observations in Constantinople indicate the reverse. Rickets does not occur among the natives of Africa. It occurred only infrequently in Japan and China until recent years. Due to the various changes in living conditions and dietary habits of the people through commerce, rickets is becoming more prevalent.

Seasonal Variation.—Kassowitz (1884) called attention to the seasonal variation in the incidence of rickets and of tetany. He noted their increase in winter, and decline in summer and autumn. Hansemann (1906) pointed out that most children born in the fall and dying in the following spring showed marked rickets, whereas children born in the spring and dying in the fall were almost free from the disease. Schmorl (1909) stated that rickets may begin at any time, but that the highest incidence of the disease was always found to be between November and May. The percentage of cases with signs of healing increased as the summer progressed, and reached its highest point in the autumn, only to fall again in the winter. Hess and Unger (1921) have confirmed these observations.

Incidence of Rickets in American Cities.—Strongman and Bowditch (1921) reported that rickets was becoming alarmingly

frequent in the unhygienic quarters of Boston. The Italians were especially affected. The children of the Jews and Poles, although kept under equally unsanitary conditions, were not so susceptible to the disease.

Hess and Unger (1922) estimated that in large cities 50-75 per cent. of artificially-fed children have rickets. Schmorl's data (1909) indicated that 96 per cent. of children dying under the age of 18 months showed rickets. Hess and Unger later stated that if thorough examinations are made of artificially-fed children, almost all of them would be found to have a certain degree of rickets.

Hess (1922) found that about half of the breast-fed infants of a large city showed signs of rickets by the end of March—the peak of the rickets season. He stated that breast milk provides but a scant factor of safety against rickets and that an additional protective influence is needed, viz., light, and that in London and New York the diet of the babies is far more adequate than the supply of light. He believes that it was lack of sunlight which brought forth the epidemic of rickets in the large cities of England which led to Glisson's classic investigations in the seventeenth century.

Eliot (1926) found that 90 per cent. of the babies of New Haven showed evidence of slight rickets by Roentgen-ray examination before they were 6 months of age. Cod liver oil and sun-bath treatments prevented entirely the development of any signs of rickets. A control series showed 18 per cent. of moderate rickets at 10 months, 25 per cent. at 13 months, and 37 per cent. at 25 months of age. She believes that in the temperate zone rickets is a nearly universal disease among infants, whether breast-fed or artificially-fed. If prevention is to be brought about, she says, anti-rachitic treatment should be begun in the first month of life and continued faithfully throughout two years.

Rarity of Rickets in West of Ireland.—Mellanby (1919) stated that in the west of Ireland, where the death rate is about 30 per 1000, rickets is an unknown disease, whereas in poor urban districts of England where rickets is rife, the death rate in children varies from 100 to 300 per 1000.

In the west of Ireland much land is unsuitable for agriculture, and cattle and sheep raising are important. There are few places where the number of cattle in proportion to the population is greater than in Ireland. Wherever it is hilly and unfit for farming, or where the soil is so thin and poor that it is suit-

able only for the grazing of animals (Northern Scotland, the Hebrides, Northern Scandinavia, Western Ireland, Balkan regions, Central Asia, and the semi-arid regions of Africa and Asia and Asia Minor) good physical development is characteristic of the people, and rickets is rare. A lack of transportation facilities makes it necessary for people to depend upon locally-grown food products, and promotes the home consumption of dairy products, but it prevents the introduction of milled cereals, sugar, molasses, and the other ordinary food products found in our grocery stores. In other words, the diet consists of natural foods,—factors safeguarding health, instead of the refined foods to which we are accustomed.

In cities in or near such regions, skeletal defects may be found, due to the similarity of the food supply to that of the people in industrial regions. Where agriculture thrives, and especially where the growing of cereal grains and tubers is the most profitable form of agriculture, it is found that stunted growth, physical inferiority and defective and carious teeth, are likely to characterize the people of both the rural districts and the cities. This point is emphasized by the prevalence of rickets in most parts of the United States and in the industrial regions of Europe where people are deriving their food supply largely from refined cereal products, such as white flour, corn meal, rice, and corn grits; and from tubers, roots, muscle meats, and sugar.

Rarity of Rickets in Island of Lewis.—Mellanby (1920-22) discussed the living conditions of the people in the Island of Lewis in the Hebrides. These observations are of interest in showing the greater importance of nutrition than of hygienic factors in promoting health, in reducing the infant mortality and in preventing skeletal defects. The diet of the natives consists in great measure of cod heads stuffed with cod livers, milk, fish, turnips, oat meal, and potatoes; it is in great measure a carnivorous one, supplemented with small additions of cereal, and tuber and root vegetables. The inhabitants live under very bad hygienic conditions. Many of the homes have a filthy byre in one end of the building. Most of the houses do not contain a window, or if they do, it is fixed. A peat fire is kept constantly burning, the smoke of which can escape only through the thatched roof or through the open door. The babies are taken out of doors only in bright weather; they are deprived of sunlight most of the time, and breathe an atmosphere sufficiently smoky to make inflamed eyes the rule. Regardless of these

unhygienic conditions the children remain practically free from rickets. The infant mortality in the Islands has not infrequently fallen as low as any place in the British Isles. The influence which counteracts the bad hygienic conditions is the universal practice of breast-feeding. Although the mothers' diet is unattractive, it is a highly satisfactory one when evaluated on the basis of its biological quality as shown by investigations on individual food-stuffs.

Studies in Inorganic Salt Utilization.—Many investigators have desired to learn whether or not a mammal can utilize the inorganic elements essential in nutrition when these are supplied in the form of inorganic or mineral salts such as the chlorides, phosphates and carbonates of the alkali and alkaline earth metals. Experiments by Kunkel (1895) showed clearly that addition of inorganic salts of iron to the diet of animals fed only milk which is too poor in iron to meet the needs of a growing animal after the store of it in its body at birth is exhausted, caused the hemoglobin content to rise far above that of animals on the same diet without iron salts. Prior to 1909 one of the questions which occupied the attention of physiological chemists was whether or not inorganic phosphorus could be utilized by an animal for the formation of nucleic acids, lecithin, and phosphorized proteins such as casein of milk. McCollum (1909) demonstrated that inorganic salts of phosphoric acid could be utilized for every physiological purpose. In recent years successful experiments involving growth conducted by Osborne and Mendel (1918) and by McCollum and Simmonds, and others, have demonstrated that inorganic compounds of sodium, potassium, calcium, magnesium, chlorine, iodine, and phosphorus, may be assimilated by animals when no other sources of these elements are provided. Sulphur must, however, be furnished in the form of one of the digestion product of proteins, known as cystine. It has been recently shown by Hart and co-workers (1928) that iron is assimilated only in the presence of copper.

Utilization of Inorganic Elements from Food-stuffs.—Courtney, Fales and Bartlett (1917) expressed the view that adding green vegetables to the diet of infants favorably influenced their metabolism; they concluded that spinach was the best one. Givens and Mendel (1917) found that the calcium in milk was more effective than soluble calcium lactate in producing calcium retention. McClugage and Mendel (1918) showed

that when carrots and spinach were added to a diet of cracker meal, meat, and lard, the calcium of milk was better utilized than that in calcium carbonate; the calcium in spinach and carrots was poorly assimilated as compared with that in milk. They concluded that green vegetables should not be used extensively as a substitute for milk as a source of calcium.

Sherman (1918) studied American dietaries and found they contained less than he considers the optimum amount of calcium (0.67 gm. per man per day). He accepted 0.45 gm. as a minimum on which a balance could be maintained. He stated that for every 100 gm. of protein the diet should furnish 1.0 gm. of calcium. He calculated the calcium content of 224 presumably typical American dietaries from the records of the United States Department of Agriculture and from those of the New York Association for Improving the Condition of the Poor. He found the average protein content to be about 1.06 gm. per kilo per man per day, and the calcium content to be about 0.74 gm. He thus found the protein to be 140 per cent. and the calcium 64 per cent. over the minimum requirement as deduced from his studies.

Sherman's studies were based upon quantitative observations on the calcium intake and output of human beings over a relatively short period of time, but they doubtless represent essentially the existing conditions among the people studied. They should not be regarded as proof that the minimum calcium intake found necessary to prevent loss of this element from the body represents the need for the optimum well-being or a safe limit with respect to this element. The optimum amount of calcium in the diet of the adult is still unknown, and from such data as are available on the effects of the relation between the amounts and proportions between calcium and phosphorus on metabolism, it seems that this will be determined in part by the phosphorus and vitamin D content of the diet. There is much reason to believe that any diet would be better if it contained more calcium in actual per cent. of the element than of phosphorus,—and this the average American diet does not do unless the calcium-rich foods are abundantly supplied. Hoobler (1911) found that a 7 kgm. infant requires 0.143 to 0.215 gm. of calcium oxide per kilo. One pint of cow's milk furnished nearly one gram of calcium oxide.

Holt and co-workers (1920) found the best absorption of calcium when the latter bore a definite relation to the fat intake.

The most favorable relation was 0.045 to 0.060 gm. of calcium oxide per gram of fat when the intake of fat was ample, i.e., not less than 4 gm. per kilo. An excessive calcium intake did not increase calcium absorption; the excess was excreted.

Human milk contains about one-fifth as much calcium as does cow's milk (Holt, Courtney and Fales, 1920). The average absorption of calcium oxide by breast-fed infants is 0.06 gm. per kilo of body weight; this equals 0.0428 gm. of calcium. Healthy infants fed cow's milk absorb 0.09 gm. per kilo per day (0.0643 gm. of calcium). Babies taking various modifications of cow's milk absorbed only 45 per cent. of their calcium intake. It becomes necessary then to give them a minimal intake of 0.13 gm. of calcium oxide per kilo per day to insure the absorption of the amount corresponding to the low average of breast-fed infants.

Sherman and Hawley (1922) found that on an ordinary mixed diet containing 750 gm. of milk daily and furnishing a total of 0.74 to 1.02 gm. of calcium per day, children 3 to 13 years of age stored 0.15 to 0.62 gm. of calcium per day, the amount being approximately proportional to the size of the child and averaging 0.01 gm. of calcium per kilo of body weight per day. When the daily allowance of milk was 1000 gm. the storage of calcium increased. Their results indicated that optimum storage of calcium is made when the diet contains one quart of milk per day for each child. With a normal allowance of other foods this will usually mean a daily intake of at least 1 gm. of calcium for the child. Children do not seem to utilize the calcium of vegetables as efficiently as that of milk. In the experiments reported the calcium balances were more variable and always less favorable when vegetables replaced about half of the milk as a source of calcium.

Blatherwick and Long (1922) found calcium and phosphorus from vegetables capable of meeting the maintenance requirements of man. Cabbage, celery, squash, spinach, lettuce, and asparagus were used. Rosemann (1922) described metabolism experiments which proved that 0.715 gm. or in another case 1.43 gm. of calcium oxide taken as calcium chloride solution daily were entirely absorbed.

Bogert and co-workers (1922) stated that the addition of yeast to a practically vitamin-free diet of lean beef, rice, skim milk, white bread, sugar, corn starch, and purified fat from nut margarines, led to a lowered excretion of calcium in normal

women. Substitution of an equal weight of butter fat for the margarine also resulted in a decreased calcium elimination. Rose (1920) stated that the calcium of carrots was utilized as effectively as the calcium of milk. Rose and MacLeod (1923) found that when blanched almond containing between 0.132 and 0.223 per cent. of calcium contributed about 73 per cent. of the total calcium of the diet, the conditions in the digestive tract may not be quite so favorable for utilization as when carrots or milk form the main source of calcium.

Rickets in Breast-Fed Infants.—A few years ago everyone would have agreed with the statement that any breast-fed child would be satisfactorily fed. The fallacy of this statement is now well known because it has been demonstrated that the diet of the mother determines in great measure the quality of the milk which she is able to produce. Prenatal conditions also determine in great measure the physiological perfection of the young.

In a foundling institution in Vienna two infants were observed; the first was born of a healthy mother, and the second of a mother suffering from osteomalacia. The mother of the first acted as foster mother to the second. With the exception of the earlier weeks of life when both infants were nourished by their own mothers, they received identical diets, consisting of breast milk from the healthy woman, supplemented by one or two bottles of cow's milk daily. The children were born in July and August respectively; by the middle of December severe rickets was observed in the child of the mother who had suffered from osteomalacia, although it had been nourished in the same manner as the baby of the healthy woman. The baby from the healthy mother was in good condition. In spite of treatment with cod liver oil, the stigma of rickets persisted until the following March, when the child was about 9 months old. It seems certain from both human experience and animal experimentation that the young born of a mother whose vitality is depleted may be predisposed to rickets (Korenchevsky, 1922).

Relation of Breast-feeding to Incidence of Rickets.—Rickets was formerly more common among the poor than the well-to-do. It has always been more prevalent among bottle-fed than among nursed infants. Recently many cases have been reported among breast-fed infants, especially among negro infants. The following table from Ferguson (1918) shows the relation of rickets in the children of Glasgow to the length of time nursed in months.

TABLE XXIII

	Marked Rickets (250 Children)		*Slight Rickets (200 Children)*		*Non-rachitic (200 Children)*	
	No.	*Per Cent*	*No.*	*Per Cent*	*No.*	*Per Cent*
Not at all.....	58	23	32	16	25	12.5
1 month	9	4	6	3	7	3.5
2 months	12	5	5	2.5	3	1.5
3 months	12	5	9	4.5	6	3
4-6 months	17	7	12	6	10	5
7-9 months	17	7	25	12.5	33	16.5
10-12 months ..	54	21	57	28.5	66	33
Over 12 months	70	28	54	27	49	24.5

This table shows that breast feeding is not necessarily satisfactory for an infant. The diet of the mother determines her capacity to produce milk of good quality. Ferguson (1918) described two diets which she believed typical of what she termed rachitic and non-rachitic families in Glasgow. These are reproduced in tabular form.

TABLE XXIV

AVERAGE CONSUMPTION PER "MAN" PER DAY OF THE CHIEF ARTICLES OF DIET IN GRAMS

(A) RACHITIC FAMILIES. (B) NON-RACHITIC FAMILIES.

	(*A*)	(*B*)		(*A*)	(*B*)
Flour.....	387.9	376.2	Other cereals.....	15.6	26.9
Potatoes.....	291.0	236.8	Margarine or butter..	32.6	38.5
Milk.....	256.0	309.0	Fish.....	15.7	35.9
Meat.....	89.1	92.6	Eggs.....	15.1	30.4
Sugar.....	91.4	84.0	Cheese.....	6.7	8.2
Oatmeal.....	40.4	36.0			

It is very significant that the quantities of milk, eggs, cheese, fish, and butter contained in diet B, over that in A, were effective in preventing rickets. Ferguson did not consider the differences in diets sufficient to indicate that the diet was of importance in producing rickets. Neither of them is of very good quality for promoting growth or for maintaining health, although B is dis-

tinctly better than A. Children restricted to diet B would also be in a borderline condition of nutritional instability, and the conditions under which one lived would determine whether or not defects definitely attributable to malnutrition would become apparent. Light, exercise and other hygienic factors, as well as occasional opportunities to secure food of special value in a supplementary way, may turn the balance one way or the other. To the casual observer the evidence may appear conclusive that the diet is entirely adequate, whereas to the nutritional expert it would be evident that there is a nutritional defect.

Relation of Diet to Composition of Mother's Milk.—Hess and Unger (1918) studied the diet of the negro women of the Columbus Hill district in New York City. They lived in great measure on milled cereal products, tubers and muscle meats. Prior to the study nearly all of the children developed rickets, but when cod liver oil was given rickets was prevented. Courtney (1923) discussed the effects of inadequate diets on the inorganic salt content of mother's milk. The continued use of a deficient diet by the nursing mother may have serious effects on the child, especially in the later stages of lactation when the mother's reserve has become depleted. The following is given as an example of a diet which was common among the women studied: one or two fried eggs; meat (usually beef) three times daily, although occasionally pork shops were eaten; about two slices of bread with no butter; two servings of potato with sauerkraut once a week. There was an entire lack of milk, butter, and fresh fruits and vegetables other than potatoes and sauerkraut. Our knowledge of the biological values of foodstuffs makes it clear that a mother could not succeed in nursing her infant on such a food supply.

Gebhart (1924) reported the results of an attempt made by the New York Association for Improving the Condition of the Poor to try to induce mothers to give cod liver oil to infants during the three winter months. This resulted in a reduction of at least 70 per cent. in the incidence of rickets in a district where it was practically universal. Suzuki (1924) found in Manchuria that rickets was more prevalent in breast-fed than in artificially-fed infants.

Chick's Observations in Vienna.—Chick and co-workers (1922) reported from their studies in Vienna that rickets developed during the winter and spring in children under hygienic conditions when receiving a diet of fresh milk to which sugar was

added, but was prevented by giving cod liver oil, more milk and less carbohydrate.

History of Use of Cod Liver Oil.—Guy (1923) gives the history of the use of cod liver oil as a remedy. In Greek and Roman literature she found no mention of the oil from the various species of Gadus. Cod liver oil has been used by the fishermen along the coasts of northern Europe for many years. In Shetland and the north of Scotland generally the liver of the cod has long been considered a special delicacy and was given to invalids and people in poor health, particularly for "old pains"; the fresh oil from livers was used as butter and was considered delicious. The use of cod liver oil by physicians was first reported in England by Darbey at the Manchester Infirmary in 1789. It was apparently used mainly as an external remedy for many years. Its recognition as a curative agent in rickets cannot be traced to any person, time or place.

"Purdah" and Rickets.—Hutchison and Shah (1922) reported a study of 2,000 children averaging up to 12 years of age who were the sons and daughters of agricultural people, artisans and laborers in and about the town of Nasik, India. Many of them had a scorbutic taint, but few showed evidences of rickets. The statement has been made that rickets is a rare disease in this district. Its existence has been clothed and escaped detection due to the fact that it occurred mostly in well-to-do Hindu and Mohammedan families. Late rickets has not been recognized by the physicians in this locality. The condition has been treated as malaria or rheumatism under the name of "haddi tap" or bone fever. The high class women live in "purdah" which they enter at 12 years of age. Infants are not taken out-of-doors until 3 to 4 months old or later if the child suffers from colds or other illnesses. When they can walk they go about like other children but at the age of 12 girls enter "purdah." This applies to the Mohammedan women of the upper caste.

The Mohammedan houses have very small rooms, and the windows are small and high, especially in the kitchens. Housing conditions are extremely bad. Privacy is the one thing emphasized. In these dark houses high class Mohammedan women spend their lives except for very short periods of respite.

High caste Hindu women are not technically in "purdah." They go about as they like but are seldom away from their homes more than half an hour daily. An infant is seldom taken out during the first 6 months and not infrequently it is a year old

before it is taken out-of-doors. A newly confined woman must not leave her room for 3 months; the child must stay with the mother and hence is not exposed to light during this time.

Hutchison and Shah emphasize that in Nasik both the Hindu and Mohammedan women have two periods in their lives when they are under the influence of "purdah," viz., in infancy and after the age of 12 years. Lower castes, composed of artisans, laborers and agriculturists, live more in the open air, and although their diet is not so satisfactory as the diet of the higher class people, the latter are subject to rickets in a more severe form and are affected more universally than the poorer people, although the children of the latter also show beading of the ribs. They also suffer from pyorrhea. The diet of the laboring classes is so defective that their outdoor life does not entirely counteract its influence.

Osteomalacia.—McCrudden (1910) described his studies on bone metabolism, especially the pathological process, etiology, and treatment of osteomalacia. In osteomalacia the bones become soft and contain much less than the normal amount of calcium salts. It occurs in animals in Europe, especially in France where it is called "cachexie ossense" or "maladie des pattes," also in southern Alabama and in parts of Mississippi and Florida. In these places it occurs especially in oxen, cows, horses, sheep, goats, swine, dogs, rabbits, and cats. Tuff (1923) observed osteomalacia frequently in cattle in Norway. Corson-White (1922) noted the occurrence of osteomalacia in several families of mammals and of birds, and in the Philadelphia Zoological Gardens this was particularly true among the Cebidae monkeys.

Seclusion of Women in India and Occurrence of Osteomalacia.—Hutchison and Patel (1921) reported a differential study of the incidence and causes of osteomalacia among the Mohammedans and Hindus of Bombay. The large preponderance of the disorder among Mohammedan women of the city is correlated with the "purdah system," which results in a lack of fresh air and exercise. They believed there was no evidence to show that osteomalacia resulted from a dietetic deficiency, since the disease is not uncommon among the wealthier class of the community. They stated that child marriage and prolonged lactation were important factors, but the sudden onset with fever in many cases suggested an infectious condition.

Osteomalacia in Northern China.—Maxwell and Miles (1924,

personal communication) and (1925) observed many cases of osteomalacia among women in China, especially in the northern part of the province of Shansi, and in parts of Manchuria. The diet of the patients consisted essentially of wheat flour, millet, bean flour, Chinese cabbage (Pai ts 'ai), garlic, mustard leaves, and salt turnip. Garlic is used as a condiment, and the amount of mustard leaves and salt turnip eaten is small. The diet is therefore derived in an extraordinary degree from cereals. Dairy products, meat and eggs are almost entirely lacking.

The disease is especially prevalent among women who have borne children, but makes its appearance frequently among adolescent girls. Cases among males are exceedingly rare. They state: "We can safely say that the food ration of the great majority of the people is deficient both as regards quantity and quality, especially the latter." When given cod liver oil, good food and sunlight the majority of the patients will slowly recover.

Instead of the calcium content in the blood of these patients being 10.5-11 mg. per 100 c.c. of serum, it ranges from 5.2 to 7.4 mg. The phosphorus of normal serum in the adult is about 3.75 mg. per 100 c.c. In 10 of these patients with osteomalacia it ranged from 1.8 to 3.8 mg.

The poverty of these people imposes upon them restrictions of diet which are comparable with those to which we have many times restricted experimental rats. These subjects, who go to extremes in trying to subsist upon a cereal diet, suffer the same type of bone changes which are seen in rats on a similar dietary regimen.

The conditions of life of the men and women differ significantly. The former spend much of their time out-of-doors and have better food than the latter, who remain in the house or small courtyard and get little sunlight or exercise. When the women have children they are long confined indoors, as are their infants in the first months of life. The children, therefore, get a poor start because of lack of outdoor life and of poor quality of the breast milk. When osteomalacia begins to affect a woman so that she feels ill, she remains indoors away from light and takes less exercise as her condition grows worse. Her habits of life tend to accentuate the severity of her condition. In these women the spine becomes bent, and the pelvic bones warp and become so greatly deformed that child-bearing is rendered difficult or impossible unless Caesarian section is performed. The long bones are bent and distorted.

Maxwell and Miles state that the poor who are compelled to work in the fields are rarely afflicted with osteomalacia. The rich, who have plenty of nourishing food with meat and fat, are seldom affected. The victims are, they say, "what we might almost call the genteel members of society; too proud to work, even if their families would allow it, not rich enough to afford the meat and fat which would correct the deficiencies in other ways." It should be emphasized that meat and fat (cod liver oil excepted) would not serve to improve this diet, as would milk and green leafy vegetables, since lack of calcium and a disproportionately high phosphorus content of the diet are the outstanding defects of the diet.

Loll (1923) analyzed the bones of persons suffering from severe "war osteomalacia" in Vienna. In every case the calcium was disproportionately high compared with phosphorus. In normal, rachitic and osteomalacic bones the calcium oxide content ranges from 51.8 to 52.10 per cent., and the phosphorus pentoxide content from 38.7 to 38.9 per cent. He found from 56.8 to 76.8 per cent. of calcium oxide and from 16.2 to 37.3 per cent. of phosphorus pentoxide. In rickets, the total ash may sink to 19.34 per cent. of the weight of the bone and in osteomalacia, to 20 to 40 per cent. There was extreme softening of the bones and consequently distortion of the skeletal structures, particularly of the thorax and pelvis.

Ash Content of Human Milk.—von Meysenbug (1922) found milk of women whose infants showed no evidence of rickets to contain on an average 4 mg. of inorganic phosphorus per 100 c.c. Milk of mothers with rachitic infants contained about the same amount. Burhans and Smith (1923) examined 54 samples of human milk. The calcium and inorganic phosphorus of the milk of the colored race were lower than that of the white, whereas the chlorine content was higher for the colored race. Debuys and von Meysenbug (1924) made 51 determinations on normal breast milk. The average was 32.6 mg. of calcium per 100 c.c. of normal breast milk, with extremes of 23.7 and 40.0 mg. Milk from mothers whose children had rickets averaged 27.5 mg., with extremes of 18.4 to 39.6 mg. The average content of calcium for white mothers was 31.1 and for colored mothers 27.3 mg.

Calcium Metabolism Studies on Infants.—Telfer (1921) found the retention of calcium and phosphorus in an 8 months' old child to be no better on a diet of skim milk and cod liver oil than on a diet of cow's milk to which butter fat was added.

Wang, Witt and Felsher (1924) reported that a change from breast milk to cow's milk in infants was invariably accompanied by a change in the quantity of calcium excreted in the feces. The quantity excreted was always greater on cow's milk with an average of 0.436 gm. excess over the breast milk. They used the same child in making these determinations. Calcium absorption and retention ran parallel, since the calcium output is insignificant. The calcium absorbed and retained is greater when a child is fed cow's milk instead of breast milk, but the percentage utilized and retained is greater for breast milk except for older children.

Acidosis as Possible Factor in Rickets.—Hodgson (1921) reported that in her experience many cases of rickets developed an acidosis at the height of the disease. Pritchard (1923) stated: "I cannot think that we are entitled to describe any case as one of rickets unless the contributory causes have definitely led to such a degree of chronic acidosis or mineral depletion that evidences of it are to be found in soft bones, muscular debility and nervous irritability." Burgess and Osman (1924) reported three cases of acute rickets in which they found a severe acidosis in which acetone bodies played no part. They interpret this to mean that the acidosis in rickets is due to one or both of the following factors: loss of bases by some other channel, e.g., feces or urine, or the presence of some other acid substance in the blood.

It should be emphasized that although acidosis may play a part in rickets when caused by lack of calcium, excessive phosphorus in proportion to calcium, and deficiency of calcifying agencies, it certainly can play no part in inducing the type of rickets which has been most studied histologically in animals, viz., that which is induced by feeding a diet deficient in vitamin D and in phosphorus, and containing an excess of calcium. The latter may be supplied as carbonate, which is a potential neutralizer of acid, and under the conditions of the experiments there was a marked excess of base over acid radicals supplied to the body.

We have pointed out the rarity of rickets in the Hebrides where the hygienic conditions were bad, but due to the satisfactory diet of the mothers, infants are protected against rickets. The Eskimo children do not develop rickets, although they live in dark huts, and for about half of the year are in the arctic twilight. They are breast-fed by mothers who eat large quanti-

ties of food rich in vitamin D, and are weaned to a diet containing much of this factor. The Negro and Italian children in American cities are especially susceptible to rickets because the pigments of their skin partially insulate them against the sun's rays; due to this fact they derive less benefit from the sun than do the fair-skinned children. The diets of the Negro and Italian children in American cities are notoriously lacking in those foods which carry vitamin D. The Negro child in Africa does not develop rickets because he lives out-of-doors and is nursed by a mother who eats food presumably of good quality, including leafy vegetables. The striking features in the occurrence and associations of rickets can be explained by the occurrence of radiant energy in the environment or of vitamin D in the food.

CHAPTER XIX

EXPERIMENTAL RICKETS

For many years there has been suspected a relationship between faulty diet and the development of rickets. Since softening of the bones is a prominent feature of the disease, lack of sufficient calcium in the diet was early thought to be the cause. Prior to 1918 much research had been directed toward the solution of this problem, but the experimental diets were not so planned that the results could be interpreted. A single example of the type of experiment planned for this purpose will serve to illustrate this fact. Dibbelt (1909) sought to produce rickets in dogs by feeding a low calcium diet composed of horse meat, carbohydrates (rice starch), and sodium chloride. This diet is low in calcium, but there are several other factors which contribute to making it a poor diet. It is low in all the known vitamins, is very high in phosphorus, and aside from the shortage in calcium, its inorganic content is not so adjusted that the results could be said to turn on a shortage of this factor. Even if the dogs had developed rickets, one could not have said that the condition was due to the absence of calcium, although this is the interpretation which would have been put upon the experiment prior to the formation of a satisfactory hypothesis concerning what an adequate diet must contain. The numerous theories which have been previously brought forward to explain the etiology of rickets will not be recounted here, since Dick (1922) and Park (1923) have discussed them in their monographs on this disease.

Rickets and Confinement.—Findlay (1908) reported experiments on the production of rickets in dogs by confinement. The diet was milk and porridge. Eight confined puppies developed rickets, whereas unconfined ones did not. He drew the conclusion that "rickets is due to the want of exercise which invariably goes along with it or is consequent upon confinement." He stated, "on clinical and experimental grounds I accordingly conclude that confinement with consequent lack of exercise is the main factor in causing the disease."

Faulty Diet and Bone Growth.—In 1917, while carrying out a series of experiments designed to discover the nature of the dietary deficiencies of two or more cereal grains, McCollum and Simmonds observed that many of their rats developed beaded ribs and showed collapsed thoraces. A systematic effort was made during the next two years to find what kinds of inorganic salt additions would supplement these cereal diets in order to prevent the abnormalities of the skeleton. It was found that no matter what salts were added, a normal skeleton was not produced unless a calcium salt was included in the diet. It was recognized that although these abnormalities suggested rickets, a diagnosis of rickets could not be made from gross bone lesions, but a histo-pathological study was necessary.

In 1919 the late Dr. John Howland was shown some of these animals. He believed some of these bone conditions were rickets. This interview resulted in a coöperative investigation between the Department of Pediatrics and the Department of Chemical Hygiene, Dr. E. A. Park and Dr. P. G. Shipley representing the former and the authors the latter.

In order to safeguard against error in the interpretation of our experimental results, we used several kinds of diets derived from food-stuffs of different origin, but believed to have like properties. One was composed of rolled oats, flaxseed oil meal, sodium chloride, and calcium carbonate in varying amounts; another was composed of four cereal grains, viz., wheat, corn, rolled oats and polished rice, with the two legume seeds, peas and navy beans; a third diet consisted of the cereal grains, legume seeds, and casein. These combinations of foods were fed with and without the addition of sodium chloride and sodium bicarbonate. Another diet used extensively was composed of steamed and dried beef liver and casein, supplemented with sodium chloride, potassium chloride, dextrin, and butter fat. It was thought that if we could produce the same histo-pathological changes in the bones on each of these types of diets and could show that they could be prevented by the addition of purified food substances of known character, the chances of error in interpreting the cause of rickets would be very small. These diets were modified in many ways by known additions and fed to young rats. Histological examinations were made of the bones, and whereas certain of the modifications produced lesions which were characteristic of rickets, as it usually manifests itself in infants, others produced lesions which had some of the charac-

teristics of rickets but differed in certain respects from the classical description of the disease as given by Schmorl (1909).

Mellanby's Studies.—While these studies were in progress, Mellanby (1919) reported the results of a series of studies on dogs designed to explain the etiology of rickets. He used the following diets:

TABLE XXV

Rachitic Diets

Diet I	*Diet II*
Whole milk, 175 c.c.	Whole milk, 175 c.c.
Oat meal.	Bread ad lib.
Rice.	
1-2 gm. NaCl.	
Diet III	*Diet IV*
Separated milk, 175 c.c.	Separated milk, 250-350 c.c.
Bread (70% wheaten) ad lib.	Bread (70% wheaten) ad lib.
Linseed oil, 10 c.c.	Linseed oil, 5-15 c.c.
Yeast, 10 gm.	Yeast, 5-10 gm.
1-2 gm. NaCl.	Orange juice, 3 c.c.
	1-2 gm. NaCl.

As a result of his studies he stated that the action of fats in the prevention of rickets was graded, certain animal fats being more anti-rachitic than the vegetable fats, and the latter differing from each other greatly. The best of the vegetable fats in preventing rickets he found to be arachis (peanut) and olive oil. He stated that his results seemed to favor the hypothesis that experimental rickets can be prevented by diets containing an abundance of the anti-rachitic factor, and that the anti-rachitic factor and fat-soluble A have a somewhat similar distribution. He pointed out that there were several points which were not in harmony with the ordinarily accepted views about vitamin A, since he found fats which do not contain this substance to be effective in preventing rickets. He stated that meat had a stimulating action on the growth of puppies far beyond its vitamin A content, and that the anti-rachitic action of meat was greater than the amount of A which it was thought to contain.

He discussed milk as an anti-rachitic food and stated that milk is undoubtedly better than the corresponding amount of butter. Later (1921) he reported further experiments showing that confinement on an adequate diet will not produce rickets in

dogs. He stated that allowing them freedom in the daytime will not prevent rickets when the diet is inadequate.

Mellanby was apparently unaware of the various factors which are essential in an adequate diet. His experimental work was not planned with a definite understanding of the dietary properties of the food-stuffs which he used. This is evident from statements like the following: "The greater the amount of bread

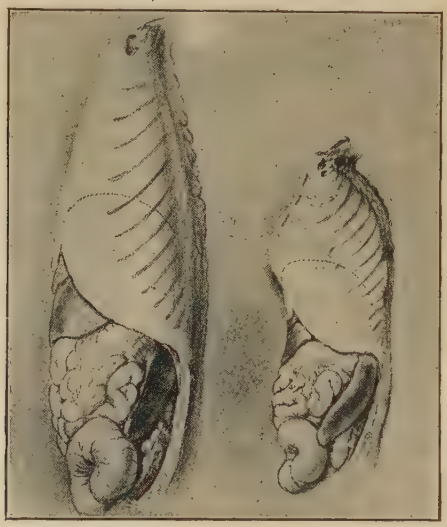


FIG. 13.—Illustrates the appearance of the exterior of the thorax of a normal and of a rachitic rat. The animals were the same age. The normal one on the left exhibits the perfection of form in this species. The rickety rat was small, round-shouldered, and had the typical "pigeon breast" seen in severe cases of rickets in children. The bending inward of the ribs resulted in a flattening and deformity of the thorax, and the formation of a groove along the line of insertion of the diaphragm.

eaten the greater will be the tendency to rickets." "Whether, therefore, the protein effect depends on its own action remains to be decided. If the anti-rachitic effect of protein is established we will be able to comprehend one reason why milk is a better preventive of rickets than the corresponding amount of butter." It will be apparent from the diets Mellanby used that he was feeding in most instances a diet low in calcium but relatively high in phosphorus. The additional calcium added in milk

together with the small amount of vitamin D aided his animals to make better bone than did the addition of vitamin D in butter fat by itself.

Mellanby, however, was the first to associate the group of fats rich in vitamin A with the prevention of rickets. Whereas some of his data were suggestive that A was essential for bone growth, some of his results indicated that perhaps another factor was needed which was not identical with vitamin A. His experi-

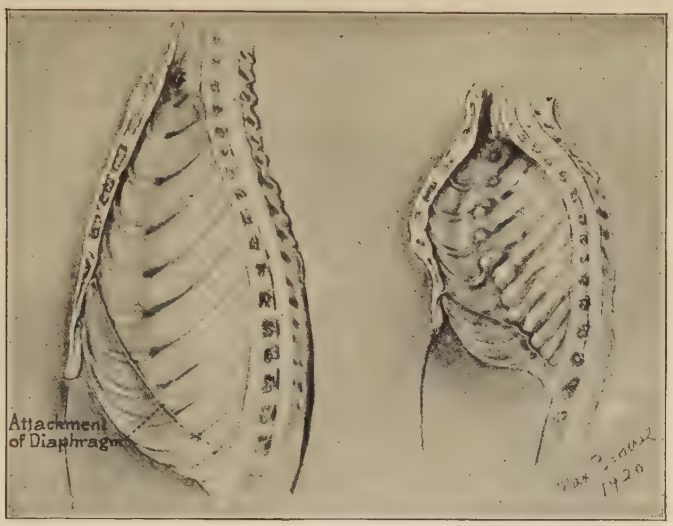


FIG. 14.—Illustrates the appearance of the inside of the thorax of a normal rat and also one suffering from rickets. Note the smooth and symmetrical form of the normal animal on the left. On the right the rickety rat is seen to have great deformity of the thorax. The shoulders are rounded and the breast bone misshapen. There are large knobs on the ribs due to spontaneous fracture and attempt at healing. The junctions of the ribs with the cartilages are enlarged, and the ribs are bent inward at their ventral extremity. The beaded condition of these is the analogue of the "rachitic rosary" seen in children.

mental work did not prove or disprove this point. This is true for the reason that he did not appreciate the importance of the calcium to phosphorus ratio in his study of rickets. His experiments brought to light the following facts: Cod liver oil possesses great anti-rachitic potency; suet and butter fat were also effective in producing calcification, whereas lard had no anti-rachitic effect; peanut oil was the most effective of the vegetable oils.

Although Mellanby's studies are open to obvious criticism,

they were important pioneer work. The dietary requirements of the dog are not well worked out and it is doubtful whether anyone could have planned experimental diets for dogs which were as clean cut and decisive as those which have been used with the rat.

Growth of Rats and Rickets.—We found that many of the little rats confined to some of the experimental diets did not grow enough to produce severe rickets. Many of the animals died in a short time because of the pronounced deficiencies of the food supplied. One of the most disturbing problems was the consumption of their fellows which were attacked and killed when the nutrition of a group was faulty. If a rickets-producing diet is one on which the animals do not grow to any extent, the condition is less marked, whereas on a diet which induces growth rickets may be exaggerated. One of the greatest problems which had to be met in investigating the etiology of rickets was the ruling out of diets on which the animals did not all perform alike. If the diet induced very little growth many of the bones presented only mild rickets, although some showed pronounced rickets; in other words, the results were inconstant under dietary conditions which produced borderline malnutrition.

Schmorl (1909) showed that the first sign of healing of rickets in rachitic children was the reforming of the provisional zone of calcification on the epiphyseal side of the metaphysis. Park and Howland (1921) proved by means of the X-ray that the administration of cod liver oil to rachitic children was followed by the deposition of lime salts in the cartilage and bones after a period of 15-21 days.

Shipley and co-workers (1921) pointed out that when cod liver oil was administered to animals with rickets a line of calcium phosphate was laid down in the matrix of the proliferative zone. This was observed on diet 2677, composed of rolled oats, flaxseed oil meal, sodium chloride, and calcium carbonate. It was hoped at the time that this diet could be used to determine the antirachitic value of various food-stuffs. It was soon discovered, however, that rats on this diet or those on diet 2638, composed of cereals and legume seeds, did not live sufficiently long or grow enough to produce a wide rachitic metaphysis free from calcified spicules. On these diets rats frequently killed and ate their companions, which also resulted in producing a line of healing. This led to confusion. The value of this reaction is dependent upon the certainty with which a given diet will produce bones in

FIG. 15.—Illustrates the marked difference in the quality of the skeleton of rats which were fed on diets which were poor and good respectively. The bones at the top (Lot 2883) are those of an animal whose diet was satisfactory except that it was too poor in calcium. Note the dark coloration due to hemorrhage; the detachment of the head from the femur; the separation of the scapula and the failure of the bone of the mandible to grow out around the base of the incisors as far as it should. Note also that these incisors are growing at wrong angle and are too long and poorly formed.

Rat 2884 took the same diet as the above but with calcium carbonate added. Its bones were much lighter in color, and were dense and well formed.

which the epiphyseal cartilage and the metaphysis are calcium-free. In other words, it is not sufficient that the diet shall produce definite rickets—it must cause an exaggerated form of florid rickets to develop without fail. One of the problems which we undertook to solve was to plan a diet which, without containing an anti-rachitic fat, would not induce ophthalmia, but which would induce sufficient growth in the animal in order to give a wide metaphysis which would have no signs of healing in it.

Sequence of Events in Growth of Bone.—The growth of a bone in length is due to a definite sequence of events which transpire at the junction of the shaft of the bone (diaphysis) with its head (epiphysis). The shaft is composed of growing bone, the epiphysis of cartilage which is largely in a resting state. As the animal grows the cartilage of the epiphysis is converted into bone through (a) encroachment on it of the bony shaft and (b) through the expansion of one or more centers of ossification which form in it. The process of growth in length of the shaft is the same which results in the expansion of the centers of ossification in the epiphysis.

The cells of the resting cartilage have no particular arrangement until they come within a short distance of the shaft or center of ossification. The cartilage cells then arrange themselves into columns parallel to the long axis of the shaft or the radii of the ossification center. This columnar zone is known as "proliferative cartilage." Lime salts are deposited in the matrix separating the cells of the thin layer of cartilage in contact with the shaft. This is known as the zone of provisional calcification. Cartilage cells in this zone lie in a matrix which is like a calcified honeycomb. As the shaft grows, tiny blood vessels sprout evenly from it and each vessel opens a single compartment in the calcified honeycomb. The cartilage cell is freed to degenerate or to be transformed into an osteoblast or bone-forming cell. Large numbers of osteoblasts follow the blood vessel sprouts into the cartilage. These osteoblasts settle on the spicules of calcified matrix which remain after the cartilage cell capsules are opened. These spicules furnish cores about which the trabeculæ of bone in the shaft are built. The osteoblasts on the spicules of calcified matrix bury themselves in a tissue known as osteoid, which they manufacture. This receives a deposit of lime salts and becomes bone.

Growth in thickness of the shaft is accomplished by cells in a thick layer of connective tissue which surrounds the shaft and

is called the periosteum. These connective-tissue cells are transformed directly into osteoblasts and lay down bone in layers beneath the periosteum. During growth, and to a lesser extent after growth has ceased, the structure of a bone is subject to constant change to meet the requirements of exercise and changing conditions of stress and strain. Old bone is constantly being removed (resorption) and new bone is always being formed. In a normal, healthy bone these processes are always in a dynamic equilibrium.

Abnormal Histological Changes in Rachitic Bones.—In the rachitic bone all these processes of growth and the maintenance of equilibrium are abnormal. In a normal bone the junction of the cartilage with the shaft forms a straight, regular line. In the rachitic bone it is ragged because there is no provisional zone of calcification and the cartilage is invaded by large tufts of blood-vessels from the shaft which destroy it irregularly. At the same time masses of cartilage persist where they should be destroyed and islands remain cut off from the main body of the tissue. Osteoid is not calcified as it is formed either in the trabeculæ or adjacent to the periosteum, and hence the bones are soft. The animal attempts unsuccessfully to compensate for the weakness due to the lack of lime salts by producing osteoid tissue in abnormal amounts, especially at points subject to stress and strain (the curved side of the bone and at the insertions of muscle and tendons). This overproduction of osteoid and cartilages causes irregular enlargement of the bones, and their weakness consequent upon the lack of lime salts results in bowing and fracture. Because of the abnormal growth at the end of the shaft, a zone known as the rachitic metaphysis forms, which consists of blood-vessels, connective tissue, osteoid, marrow elements and cartilage cells in all stages of degeneration and change into other tissue. The osteoid formed shows little sign of being absorbed in rickets as it is commonly seen. The balance of forces has swung in the direction of apposition and persistence of cartilage. In other words, a typical rachitic bone has the following characteristics: (1) there is no calcification of the provisional zone of cartilage; (2) cartilage cells persist in the shaft below the normal epiphyseal line; (3) there is an irregular invasion of the cartilage by blood-vessels and connective tissue; (4) newly formed preosseous tissue remains uncalcified and is produced in abnormal amounts—the so-called osteoid tissue; (5) formation of a richitic metaphysis which is composed of

blood-vessels, isolated masses and tongues of cartilage cells, connective tissue, and osteoid.

Importance of Phosphorus in Study of Rickets.—One of the diets (Diet 2677) which we have had most experience with produced rickets, but it also produced xerophthalmia. We were able to produce rickets without xerophthalmia, but were not able to do the reverse. Diet 2677 was composed of rolled oats, flaxseed oil meal, sodium chloride, calcium carbonate, and dextrin. We had used up to this time only sodium chloride and calcium carbonate with this diet and so had produced rickets. We now tried the addition of our 185 salt mixture to this ophthalmia-producing diet in order to see whether we could produce ophthalmia without rickets. The rats developed ophthalmia but did not develop rickets. This suggested the relation between the calcium and phosphorus content of the diet as influencing bone growth.

Phemister (1918) confirmed the observations of Wegner (1872) who fed 1/400th of a grain of yellow phosphorus daily to growing dogs, cats and chickens over considerable periods. There was new bone formation in the diaphysis which was especially marked along the epiphyseal lines, leading to the formation of a dense layer in the end of the shaft which varied in thickness with the length of the period of phosphorus administration. Phemister showed in Roentgen-ray studies the influence of phosphorus on the growth of normal bones and on the bones in the growth disturbance of osteogenesis imperfecta, and dyschondroplasia. He and co-workers later found (1921) that the end-results produced by phosphorus, and the combination of phosphorus and cod liver oil, were quite similar.

Sherman and Pappenheimer (March, 1921) published an important paper demonstrating that rickets could be produced in rats by a diet low in phosphorus, and that it could be prevented by the addition of alkaline potassium phosphate. Their diet was composed of patent flour 95, calcium lactate 3, sodium chloride 2, with or without the addition of ferric citrate. This diet regularly produced rickets which possessed the characteristics of the disease in the human being. When alkaline potassium phosphate was added to the diet in the proportion of 0.4 per cent. and an equivalent amount of calcium lactate withdrawn, the animals were completely protected against rickets. This observation clearly demonstrated that rickets can be prevented by the addition of the phosphate ion under certain dietary restrictions.

Shipley and co-workers (May, 1921) reported the production

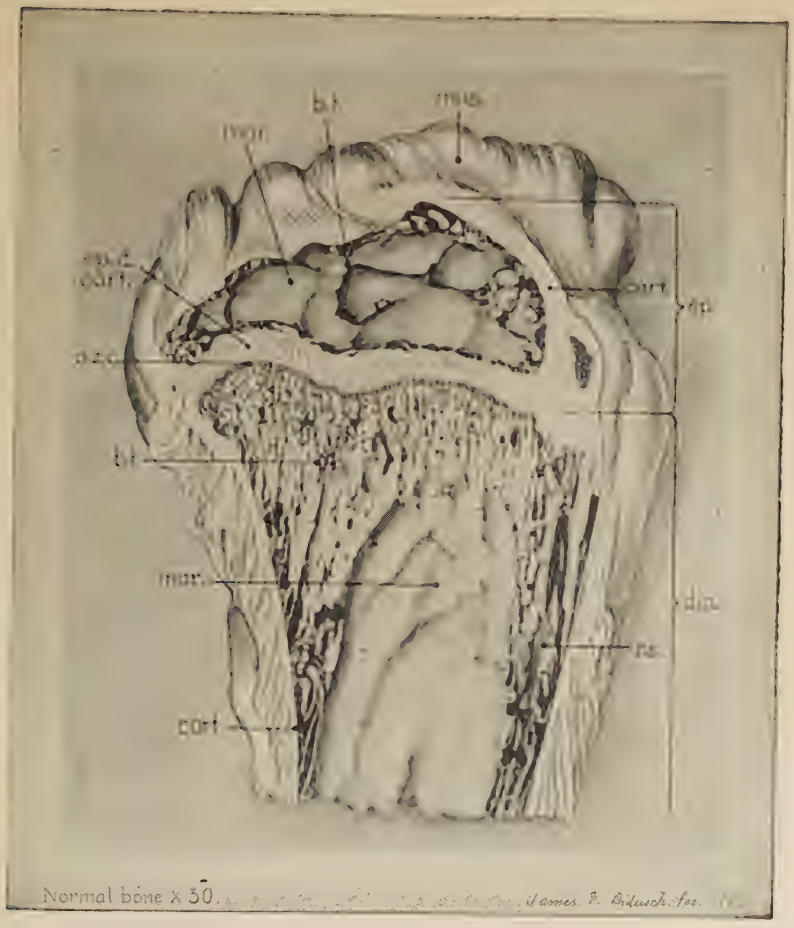


Fig. 16.—Normal bone of a rat (proximal end of tibia). This is to be contrasted with Fig. 17, which is a rachitic bone, and also with Fig. 18, which shows advanced healing. (Drawing by J. F. Didusch, reproduced by courtesy of Mr. E. M. Johnson, Jr., of the Mead Johnson Biological Laboratory.)

Legend: b.t.—bone trabeculae; cart.—cartilage; cort.—cortex; dia,—diaphysis; ep.—epiphysis; ep.d. cart.—epiphyseal diaphyseal cartilage; mar.—marrow; mus.—muscle; p.z.c.—proliferative zone of cartilage; os.—osteoid; met.—metaphysis.

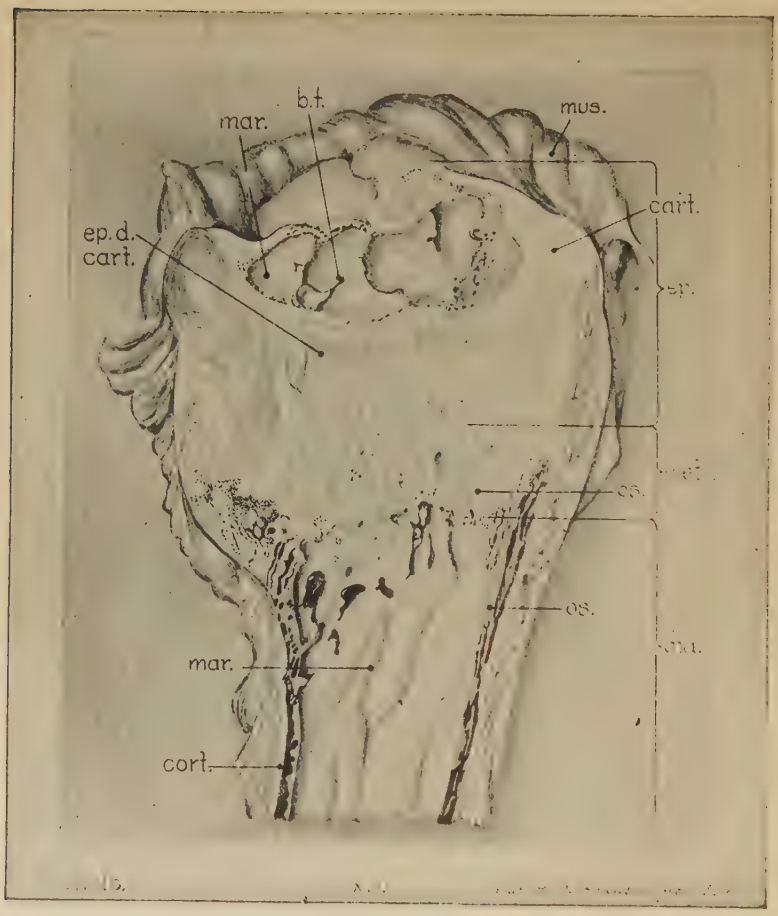


FIG. 17.—Bone of a rachitic rat (proximal end of tibia). This bone shows no healing on the diaphyseal side of the metaphysis. This shows the florid rickets produced by diet 3143. Note the enormous band of cartilage cells, the wide metaphysis which is absent from the normal bone, and the excessive amount of osteoid tissue. (Drawing by J. F. Didusch, reproduced by courtesy of Mr. E. M. Johnson, Jr., of the Mead Johnson Biological Laboratory.)

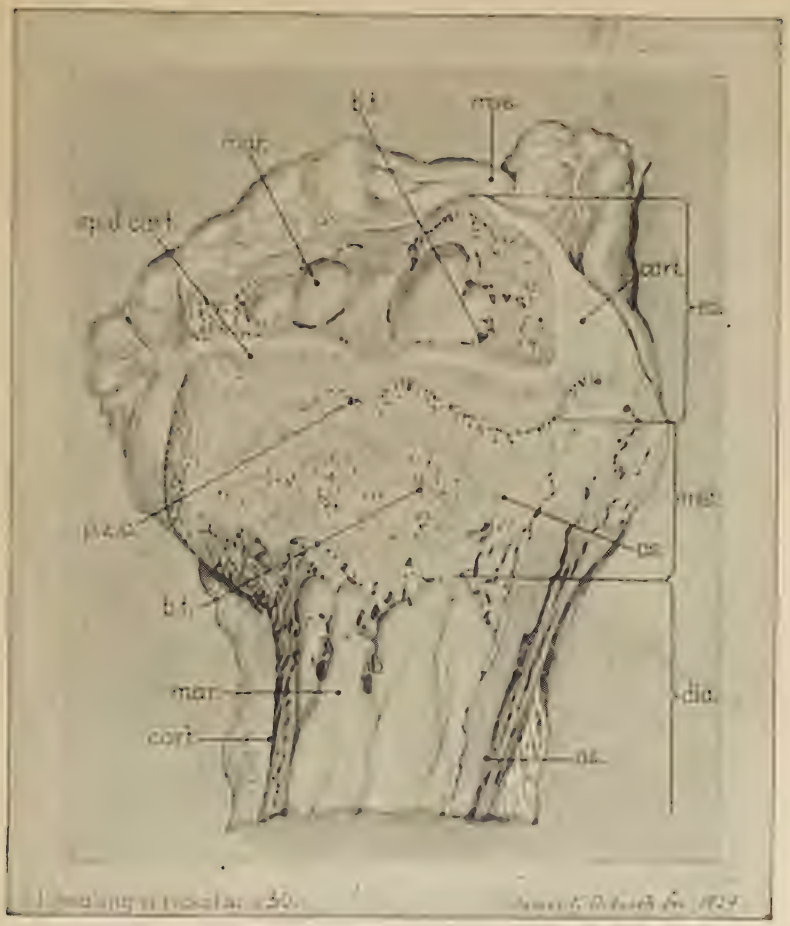


Fig. 18.—First stage of healing of a rachitic bone. When cod liver oil is administered to an animal whose bone is like that shown in Fig. 19, calcification takes place as shown at p.z.c. (Drawing by J. F. Didusch, reproduced by courtesy of Mr. E. M. Johnson, Jr., of the Mead Johnson Biological Laboratory.)

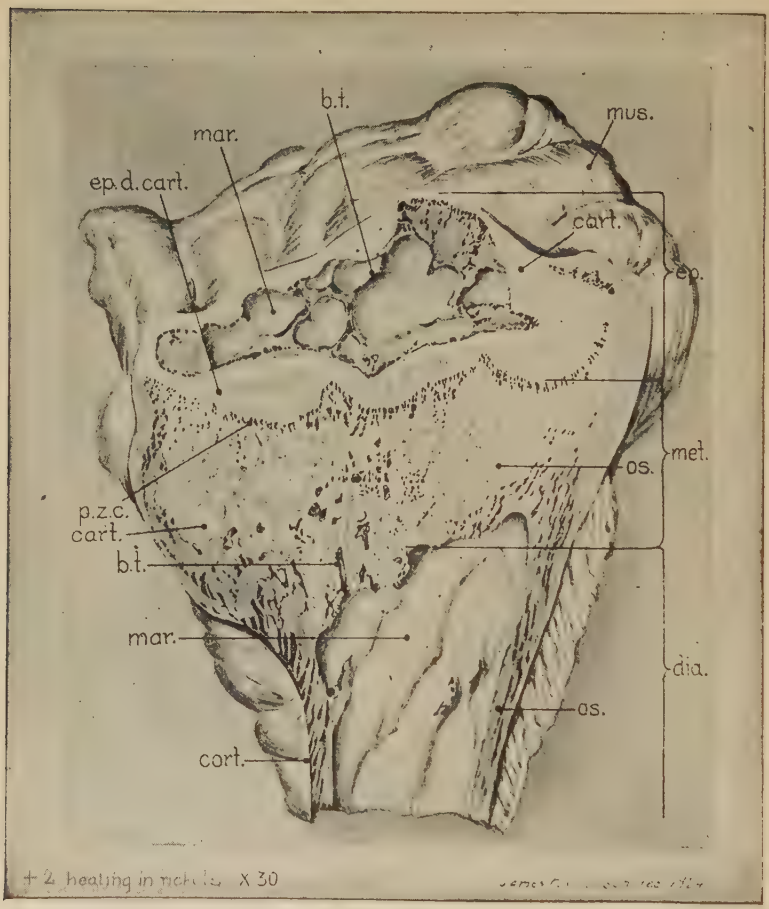


Fig. 19.—Later stage of healing of a rachitic bone than that shown in Fig. 18. The calcification is shown in black at p.z.c. When the administration of cod liver oil is continued the bone returns toward the normal structure shown in Fig. 16. If the disease is severe the bone calcifies with deformities. (Drawings by J. F. Didusch, reproduced by courtesy of Mr. E. M. Johnson, Jr., of the Mead Johnson Biological Laboratory.)

of rickets by means of two diets. That of Lot 2667 was composed of rolled oats, flaxseed oil meal, sodium chloride, calcium carbonate, and dextrin. The diet of Lot 2806 contained rolled oats, gelatin, sodium chloride, calcium carbonate, and dextrin. Both diets were deficient in vitamin A and phosphorus. When a complete salt mixture containing potassium phosphate replaced the sodium chloride and calcium carbonate, the diets no longer produced rickets, but typical osteoporosis. The observation of Sherman and Pappenheimer and of Shipley and co-workers marked a distinct advance in the investigation of rickets. They showed the importance of the inorganic constituents of the diet, especially of phosphorus in relation to the disease. With the exception of Mellanby, who had placed but little importance on the salt composition of the diet, almost all other investigators have sought to produce rickets by a reduction in the calcium. Pappenheimer and co-workers (1921) confirmed the observation that the protection against rickets in rats which was conferred by basic potassium phosphate was conferred by the phosphorus and not the potassium. They showed that in rats the absence of vitamin A does not produce rickets nor does its presence prevent it.

Low-Calcium Rickets.—McCollum and co-workers (1921) studied the effect on growing rats of diets deficient in calcium. The phosphorus content was about 0.41 gm. and 3-5 per cent. of butter fat supplied vitamin A. The bones of these rats differed in certain respects from those of rats on diets deficient in phosphorus. The metaphysis was narrow. The osteoid tissue about the trabeculæ was much more ragged in these rats than in those fed diets deficient in phosphorus. Many evidences of resorption of the trabeculæ were present. Bones which showed evidences of resorption invariably contained numbers of large cells, the cytoplasm of which was filled with large basophile granules. There were certain changes in these bones which differentiated them from the rickets seen in children. The cartilage cells retained more of their columnar arrangement in spite of a considerable invasion by blood-vessels and the evidences of bone resorption in the diaphysis were much more extensive than those seen in the high calcium—low phosphorus rickets. Shipley and co-workers (1921) stated, "whatever the defects in the salt composition in the diets employed, the effect of the addition of cod liver oil was greatly to improve the growth and condition of the animal as a whole, and to shift the pathological condition in the

bones toward the normal condition." As a result of their observations they believed it probable that the function of the organic factor in cod liver oil was to activate and coördinate cellular function. The efficiency of the organism was apparently raised and could put into successful operation defense mechanisms without which it would have been ineffectual. They believed that cod liver oil did not regulate the inorganic metabolism, but permitted the body's own regulatory mechanisms to operate with increased efficiency and economy.

Low Phosphorus-High Calcium Rickets.—Shipley and coworkers (1921) reported the production of rickets by means of a diet (Lot 3127) relatively high in calcium, and very low in vitamin A and phosphorus. The calcium was high only in the sense that it was excessive in proportion to the phosphorus content, which was extremely low. The phosphorus content was 0.209 and the calcium about 0.8 per cent. This diet produced both rickets and xerophthalmia in little rats confined to it for 5 to 6 weeks. When 0.5 per cent. of butter fat was added to this diet (Lot 3133) the animals lived somewhat longer and the pathological condition was more intensified, probably due to the slight stimulation given by the butter fat to the growth of bone.

When the level of phosphorus in diet 3127 is raised so that both calcium and phophorus are present in presumably adequate amounts so that the deficiency is limited to a lack of vitamins A and D, rickets does not develop. A deficiency in vitamin D alone cannot produce rickets. When no calcium carbonate is added to this diet so that the quantities of calcium and phosphorus are both low, rickets does not develop. When the calcium carbonate of diet 3127 was replaced by 2 per cent. of calcium phosphate so that the diet was deficient in the organic factor alone, the pathological condition which developed in the skeleton was not rickets but osteoporosis. It was such observations that led us to an appreciation of the significance of the quantitative relationships between calcium and phosphorus in the diet.

Cod Liver Oil Versus Butter Fat for Bone Growth.—Shipley and co-workers (1921) discussed the relative effectiveness of cod liver oil and butter fat for protecting the rat against an insufficiency of calcium in the presence of what under normal conditions is about a normal phosphorus supply. They pointed out that with a diet containing seeds plus casein 10, a diet low in calcium, 1 per cent. of cod liver oil was very superior to even 20 per cent. of butter fat in inducing growth and well-being in

TABLE XXVI

Composition of Diets (Percentage Composition)

No. of Diet	Wheat	Maize	Polished Rice	Rolled Oats	Peas	Navy Beans	Gelatin	Wheat Gluten	NaCl	$CaCO_3$	KCl	Flaxseed Oil Meal	Butter Fat	Dextrin	Salts 185
2667...				40					1.0	1.5		8.3		49.2	
2806...				40			10		1.0	1.5				47.5	
3120...				40			10							46.3	3.7
2638...	30	30	10	10	10	10									
3127...				40			10	7	1.0	2.0	1.0			39.0	
3133...				40			10	7	1.0	2.0	1.0		0.5	38.5	
3143...	33	33					15	15	1.0	3.0					

the animals. They were not willing at that time to make the statement that a separate factor distinct from vitamin A was to be found in cod liver oil. They stated, "We have not found it possible to demonstrate experimentally a difference between the effects of cod liver oil and butter fat when the content of the diet in calcium and phosphorus is near the optimum, the other elements in the diet being satisfactory." They further stated: "These results suggest the possibility that a dietary essential distinct from the anti-ophthalmia substance (vitamin A) may exist. If this is the case, this would appear to be present in butter fat in small amounts, but to be very abundant in cod liver oil." Hess and co-workers (1921) attempted to produce rickets in rats on a diet deficient only in vitamin A. The rats did not develop rickets, but did develop xerophthalmia. These investigators used a complete salt mixture. They apparently were not aware that rickets could not be produced in animals fed a diet in which the calcium and phosphorus contents were near the optimum.

Line Test Diet 3143.—Our experience had shown that a diet of cereals and legume seeds plus casein 10, plus calcium carbonate 3 per cent., induced rickets in animals. We fed another group of animals the diet but increased the casein to 20 per cent., leaving the calcium carbonate at 3 per cent. These animals grew well and had normal bones. We had at this time made the observation, as had also Sherman and Pappenheimer, that the phosphate ion under certain conditions would protect against rickets. Since casein is a phosphorus-containing protein, we decided to replace it in this diet by the non-phosphorized proteins gelatin and wheat gluten, leaving 3 per cent. of calcium carbonate in the diet as before. The resulting diet, diet 3143, has the following composition: Wheat * 33.0, yellow maize 33.0, gelatin 15.0, wheat gluten 15.0, sodium chloride 1.0, and calcium carbonate 3.0 per cent. Since the diet contained considerable amounts of vitamins A and B, and proteins of good quality, the animals grew and remained in a fairly good state of nutrition for varying periods depending on the season of the year. After about 18 days † on this diet (Lot 3143) the animals do not have full control of the hind legs. They have a peculiar gait due to a partial loss of control of the posterior extremities. The gait is tottering

* Hard wheats are generally much richer in phosphorus than soft wheats. Soft wheat should be employed in making this diet.

† Animals should be kept in a dark room when this diet is used.

and the hindquarters waver slightly from side to side. When the animal starts to move off rapidly it hops, usually favoring one hind leg. This diet always produces a florid rickets, and calcification of the cartilage is wanting. This diet produces a much more exaggerated picture of rickets than is seen in human beings. If this diet is fed with 2 per cent. of calcium carbonate instead of 3 per cent. the histo-pathological picture is identical with that seen in the rickets of children. Diet 3143 differs from diets 3127 and 3133 in having a higher calcium to phosphorus ratio than either. Diet 3143 induces much more growth in the animals than either of the latter two diets.

Diet 3143 produces the following changes in the bones of rats: The bones are very soft. The ends are very much enlarged. The enlargement of the ends of the long bones is due to an increased depth and probably also to an increased breadth in the epiphyseal cartilage, and to the presence of an enormous metaphysis. The cartilage in every animal is entirely free from lime salt deposits. Some irregular lime salt deposition is sometimes present in the metaphyses of certain animals. The cortex is thin; it is composed of bone with scanty central cores of calcified material with broad osteoid borders. The breadth of the osteoid borders is extreme in these animals. Few osteoblasts, or at least cells which could be identified as such, could be found lining the osteoid trabeculæ. The evidences of resorption of the calcified portions of the trabeculæ were slight. It will be recalled that in those animals suffering from low calcium-high phosphorus rickets, a considerable degree of resorption was taking place.

As has been pointed out, a rachitic bone does not form a provisional zone of calcification, since the animal fails to deposit lime salts in the inter-cellular substance of the cartilage. The proliferative cartilage persists in some places and in others is destroyed in an irregular way by aberrant capillaries which sprout in a disorderly fashion from the parent vessels in the shaft and invade the cartilage in all directions. The line of junction of the epiphysis and shaft becomes very irregular and long tongues and islands of cartilage cells are left isolated in the metaphysis and diaphysis. At the same time the bone is not formed normally either as regards the direction of the trabeculæ, since there is no calcified intercellular substance to serve as a guide for the activity of the osteoblasts, or as regards calcification, since calcium salts are not deposited in the osteoid

tissue. Osteoid is formed in excessive amounts, perhaps as an attempt to compensate for the loss of strength of the bones from failure of the pre-osseous tissue to calcify.

Starvation and Healing in Rickets.—Animals which show this wide metaphysis free from calcification are ready to be used to determine the presence or absence of vitamin D in food-stuffs. They must at this point be kept in individual cages in order that the food consumed can be recorded. McCollum and co-workers (1922) demonstrated that animals in this condition will produce a positive "line test" if allowed to starve. Howland and Kramer (1922) showed this calcification to be accompanied by changes in the calcium and phosphorus in the serum. They found serum of rats on diet 3143 to contain about 10.5 mg. of calcium and about 3 mg. of phosphorus per 100 c.c. After a few days' starvation the calcium was markedly lowered, whereas the phosphorus may be increased to 16 mg. per 100 c.c. of serum. This was earlier thought to produce the healing seen in starvation of rachitic rats.

Calcification in Vitro.—Shipley (1924) made the fundamental observation that a bone from a rachitic rat begins to calcify within 48 hours when placed in the serum or plasma of a normal rat or of a rachitic rat during the healing process. This interesting observation seems to establish that the calcification of cartilage and bone depends, in part at least, upon the exercise of cellular activity as well as on the composition of the tissue humors and that it may be independent of the arrangement of the local blood-vessels. Shipley, Kramer and Howland (1926) have further studied the *in vitro* calcification of slices of cartilage and bones of rachitic rats when these are kept in normal serum or in aqueous solutions containing calcium salts and inorganic phosphate. The calcification is delayed by the addition of egg albumen and is inhibited by heat, cold, and by protoplasmic poisons. Sliced cartilage from rachitic rats starved 24 hours calcified perfectly when kept in a basal solution containing NaCl in concentrations varying from 0.0085 to 0.0514 mol. Cartilage from unstarved rachitic rats calcified incompletely in a solution containing an appropriate concentration of calcium and phosphate ions to which sodium chloride had been added in 0.0343 mol., and not at all when the sodium chloride content was raised to 0.0513 mol. concentration. It appears from these observations that calcification of rachitic bones of starving rats takes place by some other means than that of raising the concentration of

the blood phosphate. These studies are extremely important in relation to the view held by Robison and Maitland (1927) who postulated that a bone phosphatase capable of acting upon hexose phosphoric esters is a primary factor in bone calcification. Shipley and Holt (1927) have reported further studies in this field. Kramer, Shelling and Orent (1926) found, in *in vitro* experiments, the optimum pH for calcification of rachitic cartilage in inorganic solution in which the product of Ca X P was 50, to be 7.25 to 7.30. Calcification was absent when the solution was more acid than pH 7.0.

"Line Test" Procedure.—In testing animals for the antirachitic properties of food-stuffs, it has been our custom to examine the distal end of the left femur and the proximal end of the left tibia for reformation of the provisional zone of calcification. The bones are divided longitudinally with a sharp scalpel and one-half of each is fixed in 10 per cent. formaldehyde, decalcified in Muller's fluid and imbedded in parloidion. The other half of each bone is washed well in distilled water, blotted dry and immersed in 0.5 per cent. silver nitrate and exposed to the sunlight or to the light of an arc light and studied through a binocular microscope.

Rats which give a positive "line test" differ from the controls in having a broad linear deposit of calcium salts on the metaphyseal cartilage. The band, which may not be complete, is separated from the shaft of the bone by the depth of the metaphysis and from the nucleus of ossification of the epiphysis by the depth of the epiphyseal cartilage. It can be seen on a freshly cut surface of an untreated bone as a yellow line which marks the epiphyseal border of the metaphysis. The deposit is blackened by 0.5 per cent. silver nitrate in fresh gross specimens. It appears like a cross section of a black honeycomb when it is examined with a binocular microscope. The metaphysis of these bones usually appears to be congested. The calcium salt deposit is in the proliferative zone of the cartilage. It may extend completely across the bone or may be interrupted or fragmentary according to the activity of the calcium-depositing substance. It is stained brown in permanent sections by silver nitrate, or an intense blue by hematoxylin.

Is Rickets in Animals the Same Disease as in Humans?—Park (1923) regards the rickets produced in rats to be identical with that seen in human beings: the pathological conditions found to exist in the blood are identical; the rickets produced in

animals may or may not be accompanied by the symptoms of tetany exactly as human rickets may or may not be accompanied by tetany; the same remedial measures are effective in both.

There are Two Kinds of Rickets.—Shipley and co-workers (1922) found that a pathological condition corresponding in all fundamental respects to rickets in human beings can be produced by diet in two ways: (1) by diminishing the phosphorus and supplying an optimum amount or an excess of calcium; (2) by reducing the calcium and maintaining the phosphorus at a concentration somewhere near the optimum. As a result of these experiments they were led to believe that there are two kinds of rickets: one is characterized by a normal or nearly normal blood calcium and a low blood phosphorus (low-phosphorus rickets; calcium: phosphorus ratio large); the other, by a normal or nearly normal blood phosphorus but a low blood calcium (low-calcium rickets; low calcium: phosphorus ratio). The investigations of Howland and Kramer (1921) and of Kramer, Tisdall and Howland (1921) on the calcium and phosphorus content of the blood serum in rickets and tetany supported this view. These observers found that in children suffering from rickets alone the phosphorus of the blood serum is low and the calcium about normal; in children suffering from tetany complicating rickets, on the other hand, the calcium is low but the phosphorus not far from normal. They believe that tetany is an expression of the nervous tissues of an insufficiency of the calcium ion, and that rickets is essentially an expression on the part of the skeleton of disturbed relations between the calcium and phosphate ions of the body fluids. They stated that tetany is frequently associated with rickets because rickets is a disease in which the calcium ions in the body tissues and fluids are subject to variations. Tetany occurs independently of rickets just as rickets occurs independently of tetany. Since tetany may occur with a low phosphorus form of rickets, it does not serve to mark off one form of rickets from the other. Tetany is essentially associated with a low-calcium form of rickets and for all practical purposes the low-calcium form of rickets is the rickets of tetany.

Pappenheimer and co-workers (1922) confirmed the observation that rachitic bone lesions can be produced in rats by a diet containing an excess of calcium but deficient in phosphorus or vice versa. They further pointed out that inorganic salts other than calcium or phosphorus seem to be without influence upon

the development or prevention of rachitic lesions. Hess and co-workers (1922) believe spontaneous healing of rickets in rats to be due to the animals remaining at a stationary weight which lowered the phosphorus requirement and caused a rise in the phosphorus content of the blood.

Demonstration of Existence of Vitamin D.—In 1922, McCollum and co-workers presented evidence which was all but conclusive that there is in cod liver oil and butter fat a calcium-depositing substance distinct from vitamin A. They stated: "The results of this series of experiments were so consistent that we can deduce no other conclusion than that cod liver oil contains in abundance some substance which is present in butter fat in but very slight amounts and which exerts a directive influence on bone development and enables animals to develop with an inadequate supply much better than they could otherwise do. This substance is apparently distinct from fat-soluble A, which is essential for growth and which is associated definitely with the prevention of ophthalmia (keratomalacia)." Experience with diets very low in calcium but containing cod liver oil or butter fat demonstrated that 1 per cent. of cod liver oil was superior to 20 per cent. of butter fat. The possibility remained that butter fat and cod liver oil contained one and the same substance (vitamin A) but that cod liver oil contained much more of this. Zilva and Miura (1921) stated their belief that cod liver oil contained 250 times as much vitamin A as butter fat.

Coconut oil did not prevent sore eyes but with low-calcium diets it induced better growth in animals than did olive oil. There were suggestions in Mellanby's paper that some dogs fed coconut oil were in better condition than those fed olive oil.

McCollum and co-workers (1922) compared a selected list of fats in respect to three kinds of effects in nutrition: (1) They tested cod liver oil, shark liver oil, butter fat and several vegetable oils for their potency in curing xerophthalmia due to the lack of vitamin A; (2) they made comparative tests of the same fats to determine their value in promoting growth in young rats which were restricted to a diet so low in calcium that satisfactory growth was not possible without the cod liver oil effect; (3) they studied the same fats by means of the "line test" to discover their relative values for inducing the deposition of the line of

calcium salts in rachitic bones. With the data secured from these three types of tests they were able to interpret accurately the results of much of the experimental data in the literature which were confusing. Rats weighing between 35 and 45 grams were used for these experiments.

Experimental Procedure

I. Test for vitamin A: The diet used for testing the value of different fats for the cure of xerophthalmia had the following formula:

Lot 3392

	Per cent.
Rolled oats	40.0
Casein	5.0
NaCl	1.0
$CaCO_3$	1.5
Dextrin	52.5

This diet is essentially lacking in vitamin A. If 2 per cent. of butter fat or cod liver oil replaces an equivalent amount of dextrin, xerophthalmia never develops. In making the test referred to young rats of about 40 gm. in weight were restricted to this diet until puffiness of the eyelids appeared. On the addition of sufficient fat containing vitamin A at the time when the edema of the eyelids is becoming severe, the swelling rapidly disappears and the eyes return to a normal appearance within a few days. Two per cent. of cod liver oil, 3 per cent. of shark liver oil, 3 per cent. of burbot liver oil or 2 per cent of butter fat effected a prompt cure of incipient xerophthalmia. Eight to 20 per cent. of vegetable fats would not cure the eye condition after it had once developed. It should be noted that 15 per cent. of coconut oil neither cured nor prevented xerophthalmia.

Hopkins (1920) was the first to show that if oxygen was passed through heated butter fat vitamin A was readily destroyed. Mellanby attempted (1921) to make use of this means to destroy vitamin A in order to determine whether there is a distinct "anti-rachitic substance." He found butter fat of little value for protecting against rickets after it had been oxidized, whereas cod liver oil after the same treatment, i.e., heated to 120° C. for 4 hours while oxygen was passing through it, still protected his animals. He stated: "If it should happen that 4 hours' heating and oxidation at 120° C. also leaves a large amount of fat-

soluble A in cod liver oil, it will go a long way, especially when considered together with the butter results, to clinch completely the identity of fat-soluble A and the anti-rachitic vitamin." Mellanby used no method to test for vitamin A as distinct from the calcium-depositing substance, since he did not make use of the ophthalmia test for vitamin A.

We found that 2 per cent. of cod liver oil treated with a stream of air bubbles at the temperature of boiling water for 12 or 20 hours no longer contains sufficient A to relieve rats from xerophthalmia. Untreated oil under these conditions caused recovery within 5 days. Likewise, 2 per cent. of fresh butter fat under exactly comparable conditions effects the disappearance of ophthalmia in 5 to 10 days. The significance of these results in connection with the problem of the existence of a special calcium-depositing vitamin will be discussed later.

II. Tests of fats for their protective power against the effects of deficient calcium supply. For studying the influence of fats on calcium metabolism we used the following diet:

Lot 2947

	Per cent.
Whole wheat*	25.0
Whole maize	19.5
Polished rice	9.5
Rolled oats	9.5
Casein	10.0
Whole milk powder	5.0
Peas	9.5
Navy beans	9.5
NaCl	1.0
Dextrin	1.5

* All food-stuffs were finely ground.

The properties of this food mixture had been carefully determined. The proteins are of good quality and are abundant (about 23 per cent.). Its content of vitamins B and G is adequate. It is somewhat deficient in A, but not sufficiently so to induce xerophthalmia. Its inorganic content will induce skeletal deformities. It is low in calcium, but not far from the optimum in phosphorus (if calcium had been adequate). Our problem was to see to what extent, if any, the different fats increased the efficiency of the animals in utilizing the small amount of calcium at their disposal. Shipley and co-workers (1921) showed

that a diet may be so deficient in calcium as to prevent growth in young rats and that butter fat failed to protect against calcium deficiency, whereas the same diet supplemented with cod liver oil may promote good growth.

III. Tests of fats for their power to stimulate healing in rickets. The foregoing data relative to the values of several fats for relieving ophthalmia and for increasing the efficiency of the tissues in utilizing calcium when it is present in inadequate amount in the diet, did not form a safe basis for deciding whether the effects in both types of experiments were due to vitamin A or to two distinct substances. The experiments in which coconut oil was fed suggested that this oil contained a substance which improves calcium utilization; coconut oil does not contain vitamin A, which relieves ophthalmia. We therefore supplemented the data from the above tests with observations on the effects of the several fats on the initiation of the healing process in rickets. For this purpose we used the "line test." Diet 3143 has the following composition:

	Per cent.
Whole ground wheat	33.0
Whole ground yellow maize	33.0
Gelatin	15.0
Wheat gluten	15.0
NaCl	1.0
$CaCO_3$	3.0

This diet contains an inadequate amount of vitamin A, but sufficient to prevent xerophthalmia. Although it contains an excessive amount of calcium, no deposition of calcium salts takes place in the growing bones. A discussion of the effects produced in bones by this diet has been given on page 307. After rats had been confined to this diet for about 35 days the various oils were administered.*

Coconut oil was distinctly more effective as a calcium-depositing agent than any other vegetable oil tested. It was, however, inferior to butter fat or any of the fish oils tested. The oils studied and the results of the tests are given in Table XXVII. Since starvation causes the deposition of calcium salts in the bones under the conditions of the test, we kept accurate records of food consumption.

* We have found that rats confined to this diet for 18 to 21 days in the dark are in an excellent condition for testing. This does away with seasonal variation, since during the summer it takes longer to prepare them when they are kept at room light.

TABLE XXVII

Kind of oil.	*Per cent.*	*Number of days in preparatory period.*	*Number of days fat was given.*	*Number of animals.*	*Results.*
Cod liver oil.	2	21	5	6	Healing rickets.
" " "	2	25	6	2	" "
" " "	2	28	11	3	" "
" " "	2	49	5	3	" "
" " "	2	49	5	3	" "
" " "	0.2	70	5	1	Severe rickets, a few specks of calcium in cartilage.
" " "	0.4	70	5	1	Severe rickets, no healing.
" " "	0.6	70	5	1	Beginning healing of rickets.
Cod liver oil (oxidized 4 hrs.)	2	25	6	3	Healing rickets.
Cod liver oil (oxidized 12 hrs.)	2	28	11	3	" "
Cod liver oil (oxidized 20 hrs.)	2	29	10	3	" "
Butter fat.	30	21	14	2	Beginning healing.
" "	30	32	11	4	" "
" "	15	17*	12	4	3 of these animals showed occasional specks of calcium in cartilage.
" "	15	20	20	1†	Beginning healing rickets.
Shark liver oil.	2	34	11	3	Marked healing.
Burbot liver oil.	2	30	6	2	Healing well on.
" " "	2	30	10	4	" " "
Coconut oil.	20	24	15	5	Very slight evidences of healing.
Maize oil.	20	34	15	5	Severe rickets, no healing.
Olive "	20	34	15	5	" " " "
Cottonseed oil.	20	29	14	5	" " " "
Sesame oil.	20	24	15	5	" " " "

* This preparatory period was too short. The metaphyses were narrow and irregular. It was possible to mistake calcified matrix which had existed before the onset of the disease as evidence of healing.

† This animal was badly deformed and feeble after being fed the butter fat for 20 days. There was no clinical evidence that the butter fat had been at all beneficial.

The table shows that cod liver oil, shark liver oil and burbot oil were highly effective in moderate doses in causing the deposition of calcium in the bones of rachitic rats. Butter fat was effective when fed in large amounts (15 to 30 per cent.) but it was necessary to extend the time to 14 days in order to obtain even a faint calcification. Coconut oil when fed at 20 per cent. of the diet for 15 days caused the deposition of small amounts of calcium salts. Maize oil, olive oil, cotton-seed oil and sesame oil were likewise fed at 20 per cent. of the diet for 14-15 days, but in no case was there any tendency to deposit of calcium salts. Samples of cod liver oil which had been oxidized for 4, 12 and 20 hours respectively were tested for their calcium-depositing properties. The samples oxidized for 12 and 20 hours were comparable with unoxidized samples, notwithstanding they had lost their power to cure xerophthalmia when 2 per cent. was fed. They likewise gave a positive test for the calcium-depositing substance. The sample oxidized for 4 hours cured xerophthalmia and also gave a positive "line test."

These experiments demonstrated that the power of certain fats to initiate the healing of rickets depends on the presence in time of a substance distinct from vitamin A; they demonstrate the existence of a vitamin whose specific property is to regulate the mineral metabolism of bone.

Availability of Calcium Salts.—McCollum and co-workers (1922) compared calcium lactate, chloride and carbonate in the production of rickets when fed to introduce the same amounts of calcium, using their "line test" diet 3143. The chloride and carbonate produced rickets in an exaggerated form. The lactate produced rickets of the type commonly seen in children; it resembled the rickets which we have produced with diet 3143, but using 2 per cent. of calcium carbonate instead of 3 per cent. We could detect no protective influence of the chloride which contains a non-oxidizable radical, in contrast with the carbonate or lactate, which are both potential neutralizers of acid in the body. Steenbock and co-workers (1923) found that there was no difference in the ability of young rats to utilize calcium lactate, carbonate, phosphate, silicate, or sulphate.

Zucker, Johnson and Barnett (1922) produced rickets in rats by a diet of white flour, casein, calcium lactate and sodium chloride, with a trace of ferric citrate. This diet contained an excess of base over acid-forming radicals. Substitution of calcium chloride for the lactate in equivalent amounts increased

the acidity of the diet. When this was fed to rats the bones were nearly normal or showed mild rickets. They made the diet more acid by adding 2 per cent. ammonium chloride. These rats did not develop rickets. They also tried the reverse of this experiment, viz., a diet of flour and egg albumin with a suitable salt mixture. To this diet they added 2 per cent. of sodium bicarbonate to make it distinctly alkaline. These rats developed rickets. They conclude that a diet which, from the point of view of balance between calcium and phosphorus, should not lead to rickets, may do so when the acidity of the intestinal tract is lessened.

Production of Rickets by Purified Food Substances.—McCollum and co-workers (1922), Osborne, Mendel and Park (1923) and Goldblatt (1924) produced rickets in rats by diets consisting essentially of purified food substances. Bethke, Steenbock and Nelson (1923) discussed the relations of calcium and phosphorus to growth and composition of blood and bone with varying vitamin D intake (cod liver oil). They presented data on the variation of the composition of these tissues in rats of various ages. The blood phosphate and calcium are slightly higher in very young than in older animals. There is a quantitative relation between the vitamin and calcium in effecting the assimilation of the latter. They raise the question as to whether some of the beneficial results recorded in the literature on the feeding of calcium salts may not have been due to mass action of the calcium counteracting the effects of a vitamin deficiency. They found where no fat-soluble vitamins were added that the calcium of the blood was constantly depressed, but apparently the greatest depression took place when the most phosphate was added. Irradiation with ultra-violet rays in the absence of fat-soluble vitamins in the diet raised both the calcium and phosphorus in the blood.

Park, Guy and Powers (1923) have extended the studies on the effects of cod liver oil on animals fed high calcium and low phosphorus, and vice versa. Their results show that cod liver oil acts as a regulator of the mineral metabolism in so far as calcium and inorganic phosphorus are concerned. Park (1924) stated, "one may infer that a function of cod liver oil and also of radiant energy is to maintain the normal salt equilibrium of the body in the presence of salt combinations of different complexities which are continually being absorbed from the food."

Bosányi (1924-1926) showed that subcutaneous administra-

tion of aqueous extracts of normal bone marrow causes the healing of rachitic bones in the rat. The phosphorus content of the extracts was very small, so the effects could not be attributed to this element. He believes that the failure of the initial deposition of calcium in rickets is due to the arrest of the biological functions of the bone marrow through which normally a permanent deposition of calcium is insured. Normal bone marrow produces a substance which is transmitted to the tissues and which renders them capable of calcification. This in an active state can be extracted with water. This substance is not produced in the marrow of rachitic rats.

The fat had a slight protection if melted from red bone marrow, but no effect if from yellow marrow. Marrow wholly defatted protected against rickets. When filtered through collodion, aqueous extracts of marrow had no anti-richitic effect. Dialyzed extract had no effect, whereas the gelatinous remainder not filterable had a markedly anti-rachitic effect. Heating to 70° C. did not affect the properties of the extracts, but higher temperatures destroyed it. Extracts were made of liver, spleen, thymus, thyroid and pancreas, but spleen extracts only were anti-rachitic. Extracts of the spleen of a rachitic animal were inactive. Bosányi employed the "line test" procedure.

Strontium versus Calcium in Bone Growth.—Stoeltzner (1908) and Lehnerdt (1910) observed that feeding strontium led to the formation of excessive amounts of osteoid tissue in bones. Shipley and co-workers (1922) pointed out that although strontium stimulates cartilage cell production it cannot take the place of calcium in making bone. Administration of cod liver oil to animals whose calcium had been mostly replaced by strontium caused no change in the pathological picture. This is the only instance which has come under our observation in which cod liver oil has not benefited an animal when confined to a faulty diet affecting the bones. Kinney and McCollum (1923) found that the entrance of strontium into the bones was not inhibited by the presence of calcium in the diet. Lesne, Vaglianos and Christou (1924) confirm these observations.

Composition of Normal and Rachitic Rat Bones.—Buckner and Peter (1921) collected data on the mineral content of white rats. The diet used after weaning consisted of cracked yellow corn which was kept before them at all times. Three times a week stale light bread soaked in fresh whole milk was fed as the morning meal, and twice a week shredded cabbage was fed.

Laboratory tap water was used. The following data are taken from their paper:

TABLE XXVIII

AVERAGE PERCENTAGE OF MINERAL SUBSTANCES IN THE CARCASSES

Age	P_2O_5		CaO		MgO		K_2O	
Weeks	*Male*	*Female*	*Male*	*Female*	*Male*	*Female*	*Male*	*Female*
2	0.66	0.87	0.72	0.99	0.04	0.06	lost	lost
8	1.40	1.23	1.12	1.00	0.08	0.07	0.04	0.03
12	1.18	1.12	1.02	0.98	0.04	0.06	0.03	0.04
18	1.22	1.28	0.99	1.09	0.06	0.07	0.03	0.03
40	1.79	1.54	1.47	1.41	0.09	0.08	0.04	0.03

McCann and Barnett (1922) found rachitic rats to contain less phosphorus and calcium per 100 gm. of body weight than normal rats. The reduction was the same whether rickets was produced by a diet high in calcium and low in phosphorus or vice versa. Bones of rachitic rats contain a smaller percentage of total phosphorus than those of normal rats. Jackson and Carleton (1923) have compared the weights of various organs of rats suffering from rickets with those of normal rats.

Inorganic Metabolism of Rats.—Few studies have been made on the mineral requirements of the rat for elements other than calcium and phosphorus. Miller (1926) found the minimum daily potassium requirement for normal growth in male rats to be 15 mg. and in females 8 mg. The daily maintenance requirements of mature animals was not over 2 mg. He states that an increase in the potassium intake causes increased sodium excretion in the urine, but no increased excretion of chlorine, phosphorus or calcium occurred.

Fairhall (1926) compares zinc and calcium metabolism of rats under various conditions. Under conditions of alkaline feeding the urinary and fecal zinc excretion increases at first and the balance becomes negative, followed by a return to normal. Calcium metabolism is unaffected by an alkaline diet.

Richet, Gardner and Goodbody (1925) state that in experiments on dogs which were given 0.1 gm. per kilogram of zirconium, titanium and manganese respectively, no toxic action was observed. In general, titanium and more especially zirconium

showed a slightly favorable action on the nutrition. When manganese was administered daily or once in two days the effect was unfavorable but when it was administered once in three or four days it had a decidedly favorable action.

Drinker, Thompson and Marsh (1927) state that daily doses of zinc ranging from 0.5 to 34.4 mg. in the form of zinc oxide, zinc acetate, zinc citrate and zinc malate, over periods of 35-53 weeks, had no noticeable effect on the health of rats, nor did zinc accumulate in their tissues. Most of the zinc given was excreted in the feces but a small fraction was found in the urine.

Pathological Changes in Tissues other than Bones in Rickets.—Luce (1923) found that rats on a calcium-deficient diet showed a consistent enlargement of the parathyroid glands which was due to hyperplasia and cell hypertrophy; this increased progressively with the length of time the animals were confined to the calcium-deficient diet. Murray (1923) reported that with the occurrence of rickets in pups there are changes in the thyroid. Cod liver oil, he found, acted on the thyroid in some way which either prevented destruction or increased the production of the colloid.

Kauffmann, Creekmur and Schultz (1923) assert that in rickets in rats there occur abnormalities of the osseous capsule of the internal ear which may interfere with hearing.

Szily and Edestein (1923) reported the production of lamellar cataracts in young rats nursed by a mother which was fed a diet similar to the McCollum and Simmonds diet 3143. When the litter was large they found the rats to develop cataracts, whereas when the litter was small no cataracts developed. The cataracts usually developed in the fourth week. Stepp and Friedenwald (1924) attempted to repeat these experiments. They examined the eyes of rats on diet 3143 which uniformly developed rickets but not cataracts. Szily's view was that cataracts were due to deficiency of vitamin A, an excess of calcium and a deficiency of phosphorus in the diet. Stepp and Friedenwald confined animals to a diet very low in A and phosphorus but relatively rich in calcium. The animals developed both rickets and xerophthalmia but no cataracts. They conclude that diets deficient in either vitamin A or D with an excess of calcium and low phosphorus do not lead to cataracts in young rats. Dr. Friedenwald (personal communication) has recently informed us that the cataracts noted by Szily were probably the result of the eyes of very young rats becoming cold from lack of protection against chilling.

Chemical Studies on Active Principle in Cod Liver Oil.—Zucker, Pappenheimer and Barnett (1921) stated that the anti-rachitic principle was found in the ether-soluble unsaponifiable fraction of cod liver oil after alkali hydrolysis, that it is neither an organic base nor cholesterol, but possibly a related sterol. They found the fatty acids of cod liver oil inactive in curing rickets. When the calcium soaps were precipitated from aqueous solution the unsaponifiable matter including the active substance was precipitated with the soap. From the calcium soap acetone extracted the active material. Zucker's preparation was active in dilutions of 1:1000. A single dose brought about healing of rickets in the same manner as a succession of small doses. The preparation was found to be free from vitamin A. Zucker and Matzer (1924) found that when a solution of the active principle of cod liver oil is put into olive oil or glycerol, and injected subcutaneously into rats, it does not produce healing or change the pH value of the feces, provided measures are taken to prevent licking the site of injection. Kramer and Wilkins (personal communication) have cured rickets in children by the intramuscular injection of an extract of cod liver oil. The increase in the calcium and inorganic phosphorus concentrations of the serum was similar to that following the oral administration of cod liver oil.

Nelson and Steenbock (1925) reported that the anti-ophthalmic and anti-rachitic properties of cod liver oil are not precipitated by digitonin. A petroleum ether solution of the unsaponifiable fraction of cod liver oil did not lose its A or D potency in 1.5 years.

Changes in the Composition of the Blood in Rickets and Tetany.—Howland and Marriott (1918) discussed the theories concerning the etiology of infantile tetany. Tetany has been attributed to: (a) a diseased condition of the parathyroid glands; (b) the character of the food; (c) intoxication by calcium; (d) intoxication by guanidin, or methylguanidin; and (e) lack of calcium. They stated that the evidence in 1918 failed to support most of these theories, although there had been in the literature for a hundred years satisfactory descriptions of infantile tetany. They pointed out that normal calcium of the blood is 10-11 mg. per 100 c.c. of serum. In some cases of rickets they found a lowering of the calcium to about 8 mg., although in others it remained normal. In tetany during the active symptoms the calcium content of the serum was invariably reduced,

and might fall as low as 3.5 mg. In convulsive disorders other than tetany they found no reduction of calcium of the serum. Calcium chloride given by mouth caused a prompt relief of the spasmodic symptoms.

Howland and Kramer (1921) showed that in rickets there is a marked decrease in inorganic phosphorus in the serum. To this deficiency they ascribed the failure of calcium deposition. Non-rachitic infants and young children have between 10 and 11 mg. of calcium and about 5 mg. of inorganic phosphorus per 100 gm. of serum. All children under two and a half years with 3 or less than 3 mg. of phosphorus had active rickets. When cod liver oil was given the phosphorus content returned to normal and was accompanied by healing of the lesion.

Kramer, Tisdall and Howland (1921) found the calcium of the serum in a large series of cases of rickets associated with tetany to be about 5.4 mg., or approximately half the normal. In 10 cases of active tetany the average sodium content was 325 mg., which is about normal. The highest concentration was 337 mg., and the lowest 320 mg. In a similar number of cases of active tetany the average potassium concentration of the serum was 24.3 mg., which is about 25 per cent. above the average of 19.3 mg. The magnesium concentration was 1.9 mg., the average for normal infants being about 2.5 mg. It is obvious that the essential change is in the calcium and that this change is constant. The sodium is normal in amount; there may be a slight increase in the potassium; the concentration of magnesium is essentially unaltered. The phosphorus content is usually within the limits of normal or slightly above normal. An increase in the inorganic phosphorus does not seem to be responsible for infantile tetany. The ratio Na:K and Ca:Mg is increased; this is almost wholly due to a decrease in the concentration of calcium. The increased irritability of the neuromuscular mechanism which is the essential phenomenon in infantile tetany they believe to be due to the diminution in the concentration of calcium in the serum.

Von Meysenbug (1921) concluded that there is no change in the proportion of diffusible calcium in the serum in human rickets or in experimental dog tetany. These results are out of harmony with those of Kramer and Howland (1922) who report that the normal concentration of calcium in the serum of rats is from 9.5 to 10.5 mg., and of phosphorus from 7.0 to 8.5 per 100 c.c. of serum. These values are not increased by changes in the

diet or by ultra-violet light. In animals fed diets very low in calcium or phosphorus the concentration of the elements in the serum could be diminished by as much as 50 per cent. Subsequent addition of fish liver oil to the food brought the values back to normal. The phosphorus could also be raised by illumination with light of less than 3000 Å. They also found that a similar effect could be obtained by starvation for a few days.

Kramer, Casparis and Howland (1922) treated 5 children showing clinical evidences of rickets, confirmed by X-ray examination of the bones, by systematic exposure to the rays of a mercury quartz lamp. This was followed in every case by a healing of the rachitic process in the bones. Before treatment was begun the inorganic phosphorus of the serum was low, ranging from 2.7 to 3.2 mg. The changes in the phosphorus of the serum following the light treatment were the same as when the children were given cod liver oil. The pigmented skin of Negroes did not interfere with the action of the light rays; they did not require more intensive treatment than did whites to bring about the effects.

Telfer (1922-23) found no negative balances of either calcium or phosphorus in rickets. He believes there is no support for the theory that excessive decalcification is the cause of bone softening in rickets. He suggests that defective absorption of calcium from the intestine, due possibly to the result of some alteration in the gastro-intestinal functions, may be a factor in the causation of bone softening in rickets.

Howland and Kramer (1922-23) found that in uncomplicated rickets the concentration of calcium in the serum is normal or nearly so, whereas the phosphorus concentration is low, so that the product of the two concentrations is at or below 30. In tetany the product is also low on account of the striking reduction in the calcium. In all of their cases rickets was present. After cod liver oil or ultra-violet ray therapy, the phosphorus rises so greatly that the product is two or three times what it was before. The graph (Fig. 20) taken from their paper was constructed from figures for the concentration of calcium and inorganic phosphorus both of children and of rats; it illustrates the significance of the products of these concentrations. The abscissa shows the concentrations of phosphorus; the oblique lines represent the concentrations of calcium, and the products may be read off from the ordinates.

It will be noted that below the horizontal line corresponding

to the figure 30 on the ordinate all the triangles and circles are light. Above 40 they are all dark; between 30 and 40 only one is dark. This graph is to be interpreted that when the product of calcium times phosphorus is below thirty, rickets is to be expected, and when between 30 and 40 it is probable. When the product is above 40 either healing is taking place or rickets is entirely absent.

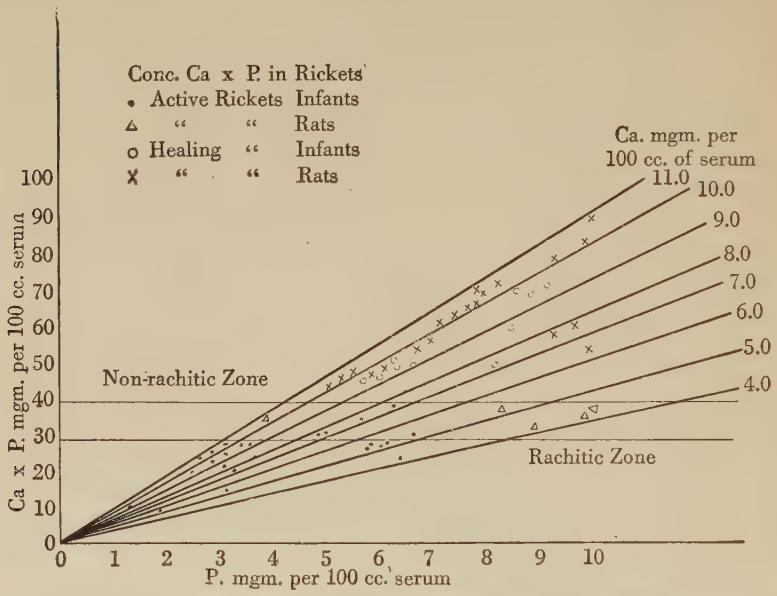


Fig. 20.—Graph taken from paper by Howland and Kramer, Monatsschr. f. Kinderheilkunde, 1923, xxv, 279.

Orr, Holt, Wilkins and Boone (1923) report metabolism studies which show that infants with active rickets fail to retain phosphorus and calcium, although adequate amounts are present in the diet. On irradiation with ultra-violet rays there was a very marked increase in the retention of these elements. They conclude that the ultra-violet rays act by increasing the absorption of calcium and phosphorus from the intestine. The healing effects of both cod liver oil and the ultra-violet rays appear to be due to their ability to promote absorption from the intestine.

Hess, Calvin, Wang and Felcher (1923) found in severe rickets that the phosphorus was markedly lowered even in those babies exclusively breast-fed, whereas the calcium was not always low-

ered. In 40 per cent. of the tetany cases severe rickets was present.

There are several explanations for the diminution in the retention of calcium and phosphorus in rickets. One is that calcium and phosphorus are not absorbed from the intestinal tract. On this supposition it is likely that either the digestive functions are disturbed in some peculiar way or else the combination of food substances is such that insoluble products incapable of absorption are formed. Another possibility is that the calcium and phosphorus are absorbed, but on account of abnormal conditions in metabolism they cannot circulate in the blood; they are either driven out of the body or are used to carry some other substances. On this latter supposition it is necessary to assume the primary disturbance to be in the metabolism. In either case it seems probable that the deposition of lime salts in the bones is determined chiefly by the product of the concentration of calcium and phosphate ions in the circulating fluid, as Howland and Kramer have indicated. Their work shows that when the concentration of calcium and phosphorus increases sufficiently deposition of lime salts occurs.

Metabolism of Calcium and Phosphorus in Rickets.—Shohl and Bennett (1928) have reported elaborate studies on the balance of calcium and phosphorus in experimentally produced rickets in dogs. Their investigations show that development of severe rickets is associated not with a negative balance but with a diminished positive balance of both calcium and phosphorus. In some cases with puppies, although the phosphorus in the diet exceeded the calcium, the phosphorus balance was smaller or even negative. This indicates an exception to the widely accepted belief that the element of which least is consumed is the limiting factor in nutrition. In rickets the limiting essential may be a deficiency of vitamin D, the action of which, they point out, constitutes a mechanism in the stabilization of calcium and phosphorus metabolism. They stress that in ossification too much emphasis has been placed upon calcium and not enough on phosphorus. The process is often referred to as calcification; but they point out that the more important defect in rickets lies in the inadequate retention of phosphorus.

Biological Testing of Anti-rachitic Preparations.—Considerable difference of opinion exists as to the most satisfactory procedure for estimating, by means of biological tests with rats, the anti-rachitic potency of oils, irradiated foods, ergosterol prepara-

tions, etc. Howland and Kramer (1921) introduced the quantitative estimation of the phosphorus and calcium content of rat serum as an index to the severity of rickets or of the effect of a curative agency. Steenbock and co-workers (1923, 1924, 1925) make use of bone ash determinations as one set of criteria by which to judge the anti-rachitic effects of certain rations. Chick (1926) also considered results of bone analyses reliable criteria in the matter of judging severity of rachitic conditions. In an elaborate study carried out by Adams and McCollum (1928) the results indicate that there is scarcely a standard behavior of animals as evidenced by bone composition. There is a general trend of events as indicated by ash values, for example, the per cent. of ash decreases as the severity of rickets increases. This decrease, however, is not by graded amounts. Individual variations are so pronounced that many animals of a given group show appreciable deviation from the average established for the group. Hence it is difficult to decide whether the comparatively slight deviations shown by test animals from the so-called average are due to cod liver oil influence or whether they are individual variations within normal limits. Their data do not lend themselves for the establishment of a "normal" of behavior either in the case of animals on the rickets-producing diet alone, or animals subjected to cod liver oil treatment. Steenbock (1925) states that data with regard to the ash of bones were much less uniform with McCollum's diet 3143 than with Steenbock's ration 2965. The method generally used, which involves cleaning, extracting, and ashing, to obtain the ash of bones may be the cause of the variations noted. Kramer and co-workers (1926) state that an erroneous impression is given when the ash of bones is used as a criterion in this work, since by this method organic phosphorus is converted to phosphate.

Several investigators have employed various pathological changes as standards wherewith to follow rachitic developments and to judge the significance of bone analyses. Gross lesions, radiographic evidence, and histological picture have been used—and late and early developments in the disease have thus been involved. Observations have been made with respect to various bones—long bones in particular—but even in this group of bones rickets is first evidenced in the ribs, and later in the femurs and tibiæ.

In view of the factors just discussed it is little wonder that various investigators should reach different conclusions. Scrutiny

of their data indicates for the most part that there is a downward trend of values for bone ash as the severity of rickets increases. Moreover, where sufficient values are given the averages drawn are seen to be based in many cases upon rather diverse figures. The conclusions drawn as to the merits of bone analysis as an indication of rachitic developments differ, seemingly, because of the interpretation given the findings, and not because the data are actually at variance.

The trend of inorganic phosphate of the blood has been taken by some (Bethke, Steenbock and Nelson, 1923-24; Dutcher and co-workers, 1925) to indicate development of rickets or progress of healing. The phosphate value was considered because it falls below the normal level in rickets, only to rise again as healing progresses, whereas the blood calcium remains at a fairly constant level regardless of the stage of the disease. The actual phosphate values are not to be correlated with any specific degree of healing nor with any specific degree of rickets. Moreover, since phosphate is deposited as the calcium salt in the calcified bone, it is more logical to consider the calcium: phosphate relationship in predicting and discussing the possibilities of calcification of the rachitic bones. That the physico-chemical relationship applying to the precipitation of this salt $Ca_3(PO_4)_2$ from solutions supersaturated with respect to it, holds in its deposition from blood serum, is quite well indicated by the work of Holt (1925). Adams and McCollum confirmed Holt in that the ion product value is greater than 8.0×10^{-25} in healing or absence of rickets, and less than this value in active rickets.

In the biological assay of cod liver oils or ergosterol preparations the basal diet must satisfy the following requirements: (1) permit of fairly rapid growth of the test animals; (2) bring about a condition of florid rickets in 18 to 21 days; (3) permit of healing of florid rickets within 8 days when cod liver oil is added to the basal diet.

Should a sample of oil fail to evidence curative effects at the 0.125 per cent. level, progressive increase of dosage would serve to establish a level at which healing would be produced. An idea of the relative potency of such an oil would thus be obtained.

Blood analyses calculated to give the ion product values $[Ca^{++}]^3 \times [PO_4^{=}]^2$ indicate whether test animals show active rickets or healing. The results of blood analyses can be correlated with "line test" (i.e., histological) observations in practically all cases. The "line test" pictures as evidenced by the

majority of a group of ten or fifteen animals should be indicative of the potency of a given oil. Cod liver oils vary with respect to their content of anti-rachitic vitamin. Using the "line test" assay it is possible to indicate only relative potency of oils. There appears to be no method at present known, by means of which absolute values can be secured by feeding tests. Quantitative absorption spectra offer a more satisfactory basis of estimating ergosterol. It appears, therefore, that no biological assay with respect to the vitamin D content of substances would yield more than relative values.

Poulsson (1928) describes a quantitative method for determining vitamin D. The chief features in his procedure are as follows: The diet of Steenbock and Black (#2965) was employed. After about 25 days a radiogram of the left knee joint was taken and the rats were again weighed. In the test period the anti-rachitic substance to be tested was administered for 6 days, after which another radiogram was taken and the rats were again weighed. The efficacy of the anti-rachitic agent is indicated in units per gram on a principle analogous to that adopted by the United States Pharmacopœia in its prescription for vitamin A.

Zucker and Matzner (1923) found the feces of rats maintained on a rickets-producing diet to be consistently alkaline but they quickly became acid when vitamin D was fed. Grayzel and Miller (1928) found this true for dogs, but Redman (1928) could not confirm the result with children.

Jephcott and Bacharach (1928) worked out a biological test for vitamin D, using as a basis the change of hydrogen ion concentration of the feces, but Shohl and Bing (1928) found that the test could not be relied upon, at least with certain diets, and it has received but little attention.

Bills (1927) made assays of a series of oils from many sources to determine their values as sources of vitamin D. The results are shown in Table XXIX.

The absence of vitamin D from the blubbers of seal and whale is remarkable, and the poor quality of oils from catfish and dogfish and some other oils is unexpected. It is remarkable also that at the period of the year when the cod are storing fat most rapidly in their livers they feed almost exclusively on caplin in certain localities. It seems improbable that the cod livers could derive all of their vitamin D content from the food of the cod. Bills (1927) fed young catfish kept in the dark solely upon veal

TABLE XXIX

SHOWING THE VITAMIN D POTENCY OF VARIOUS OILS IN COMPARISON WITH COD LIVER OIL

Source and description of oil	*No. of samples*	*Relative potency*
Puffer fish liver, supplied and previously described by Hess and Weinstock	2	1500
Cod Liver, Newfoundland medicinal	500	100
Goosefish liver (*Lophius piscatorius*), Boston	1	100
Herring, Newfoundland	4	100
Sardine, California	4	100
Menhaden, Chesapeake Bay	5	75
Shark liver, Newfoundland	1	75
Muddy catfish (*Leptops olivaris*), fatty tissues, Ohio River	1	40
Coalfish liver (American pollack), Boston	9	40
Salmon trimmings, Pacific coast	3	20
Haddock liver, Boston	5	10
Channel catfish (*Ictalurus punctatus*), visceral fat, Ohio River	1	6
Squid (*Ommastrephes illecebrosus*), Newfoundland	2	6
Caplin (*Mallotus villosus*), Newfoundland	4	3
Dogfish liver, Newfoundland	2	3
Seal blubber (*Phoca graenlandica*), adult female, Newfoundland	1	3
Seal blubber (*Phoca graenlandica*), new-born, Newfoundland	3	Nil.
Whale blubber, antarctic, Graham Land	1	"
Cod liver, commercial hydrogenated	2	"
Veal fat, extracted with ether	1	"
Oleo, commercial	1	"
Coconut, edible white	1	"
Cottonseed, edible	1	"
Linseed, U. S. P.	1	"
Maize, edible (from white maize)	1	"
Olive, edible	1	"
Peanut (Arachis), edible	1	"

muscle the fat of which was devoid of anti-rachitic properties. Fish which survived six months on this diet, having doubled their weight, contained fat with the same vitamin D content as the control fish taken from the Ohio river. Irradiation of catfish under 20 cm. of water did not increase the vitamin D content of their fat. Bills concludes that vitamin D may be synthesized by the fish.

Macy, Outhouse and Brekke (1928) found the fat of cow's milk to contain an appreciable amount of vitamin D, but that human milk has considerably less. Mellanby (1928) states that

commercial ergot, which contains ergosterol, has a powerful antirachitic effect without previous irradiation with ultra-violet rays.

Blood Studies in Fractures.—Tisdall and Harris (1922) reported studies on the inorganic content of serum of 42 persons with fractures, ranging in age from 4 months to 44 years, and of 11 persons between the ages of 20 and 57 years. The inorganic phosphorus content of normal serum from birth to 20 years was remarkably constant at 5.6 mg. per 100 c.c. of serum. At 20 years of age the phosphorus content drops sharply to about 3.75 mg. and remains constant throughout adult life.

During the period of union of fractures in the adult they found the phosphorus content of the serum to be raised to a level of about the same as that present in childhood. There was a close parallelism between the concentration of the inorganic phosphorus in the serum and the deposition of new bone, both during the process of growth and of repair. Eddy and Heft (1923) confirmed the observations of Tisdall and Harris.

Peterson (1924) reported an experimental study of ununited fractures with especial reference to the inorganic bone-forming elements in the blood serum. He applied the principles elaborated by Howland and Kramer, and by McCollum, Simmonds, Shipley and Park (p. 324) in their studies of experimental rickets. It was possible by dietetic management to lower the phosphorus content of the blood of dogs to a point where the product of calcium times phosphorus was less than thirty. In the healing of fractures a definite relationship exists between the concentration of the inorganic bone-forming elements in the serum and the rate of repair. If the phosphorus and the calcium times phosphorus product are again raised to their normal level the fractured bones will unite. This condition could be established by dietetic management. He (1924) applied these principles to a clinical study of ununited fractures. The following case is illustrative of what sometimes occurs in human fractures. On July 19, 1921, a man, aged 47, suffered a fracture of both radius and ulna. The arm was put into a splint. On August 22, 1921, no union had taken place. On January 10, 1922, an open reduction was performed and the fragments were wired. This did not result in union. On July 24, 1922, a Lane plate was applied. Infection followed and the plate was removed. On December 11, 1922, a third operation, and on March 23, 1923, a fourth unsuccessful attempt was made for union. The patient came to the Johns Hopkins Hospital in November, 1923, and was operated

upon on November 7. The ends of the fragments were freshened and wired into position. The calcium and phosphorus of his serum were found to be 10.8 and 2.225 mgm. per 100 c.c. of serum respectively.

Sample menus which the patient received are as follows: Dinner: soup, creamed salmon, baked potato, stewed prunes; Breakfast: cereal and bacon; Dinner: soup, meat loaf, mashed potatoes, string beans; Supper: soup, creamed potatoes, stewed apricots; Breakfast: cereal and bacon; Dinner: soup, roast veal with dressing, mashed potatoes, buttered peas, ice cream; Supper: creamed potato, beet salad, bananas; Breakfast: cereal and bacon; Dinner: soup, frankfurters, potatoes, buttered beets; Supper: soup, creamed chipped beef, baked potato, stewed apricots; Breakfast: cereal and eggs; Dinner: soup, stew with crust, potatoes, buttered string beans; Supper: soup, hashed brown potatoes, stewed pears; Breakfast: cereal and bacon; Dinner: soup, corned beef and cabbage, potato, chocolate pudding; Supper: soup, potatoes, apple sauce; Breakfast: cereal and hash; Dinner: soup, Hamburg loaf, creamed potatoes, corn. Bread and butter was included with all menus and milk was provided for the breakfast cereal. In addition this patient received daily 2.0 gm. of calcium carbonate, 1.0 oz. of cod liver oil, 500 c.c. of buttermilk, orange juice, or raw vegetables as cole slaw. In addition he was also given calves' liver three times a week.

No union took place during the first 6 weeks following the operation. During this period the product of the inorganic calcium and phosphorus gradually increased. On January 2 the calcium was 10.3 and the phosphorus 3.75, and the product 38.1. The mercury vapor quartz lamp was substituted for the cod liver oil, since it was thought that this would raise the phosphorus still further. On February 18 the calcium was 10.4 and the phosphorus 4.0, and the product 41.6. On May 1 the radius had united and the ulna was uniting. He cites other instances where cod liver oil was used without light treatment and where union took place. Dr. Peterson suggests that cases of non-union fractures may be essentially a dietary problem.

It will be clear that in the development of rickets several factors operate. Diet is of fundamental importance, but hygienic conditions, especially sunlight, are at times a determining factor. The absence of rickets among the primitive Eskimo demonstrates clearly that the diet of the mother during prenatal life and the nursing period can protect the child against rickets

in the absence of sunlight. Many human infants are born predisposed to rickets because of faulty diet of the mother. The long period of slow growth in the human infant puts it in jeopardy in greater degree than is the case with the young of domestic animals, since conditions favorable to rickets can act over a longer time. Outdoor life in winter is less effective than in summer because in winter the child in the temperate regions is so heavily clothed that it is but little exposed to the sunlight.

The experiments reported in this chapter demonstrate that rickets can be produced experimentally in rats by certain unfavorable relations between calcium and phosphorus and an inadequacy of vitamin D. Since rickets is a disturbance of calcium and phosphorus metabolism, disturbances of digestion which interfere with the assimilation of these elements undoubtedly contribute to the development of this disease in children.

CHAPTER XX

ULTRA-VIOLET RAYS IN PREVENTION AND TREATMENT OF RICKETS

There has long been a belief among clinicians that sunlight, calcium-rich foods and cod liver oil are of value in the treatment of disease, especially rickets and tuberculosis. Prevention and treatment of rickets with ultra-violet rays has been so spectacular that any discussion of nutrition must include the rôle played by light. Great progress has been made in an understanding of the nature of vitamin D and its formation from ergosterol by ultra-violet rays. In this chapter the principal studies in this field are presented.

Early History of Heliotherapy.—The use of light for the treatment of disease is not of as recent origin as one might think. Since the earliest times, physicians have recognized the value of sunlight in the treatment of disease. Many references to light as a therapeutic agent can be found in the works of Herodotus, Hippocrates, Celsus and Galen. Pliny stated, "Sol est remediorum maximum." The Greeks and Romans built solaria in their homes. The ancient Egyptians worshiped a Sun God, and recognized the beneficial action on disease obtained by visiting sandy beaches. As early as the fourth century A.D., the Greek physician Oribasius attempted to popularize heliotherapy. Pliny the younger also advocated the use of light. In the early literature of that time, there are indications that artificial sources of light were used. The Germans, according to their religious creeds, exposed their sick infants to the sun's rays. They believed that the sun furnished Freya, queen of the heavens, with an efficient means of combating all disease.

Avicenna, recognizing the importance of heliotherapy, adopted methods for the use of sunlight which served as a basis for our modern technic. Paracelsus was probably the first to call attention to the value of the climate of a mountainous district as a therapeutic aid. He recommended the district of Saint Moritz, Switzerland. However, considerable credit is due to Jean Jacques

Rousseau, who popularized the mountainous climate in "Novelle Héloise."

It appears that the French are really responsible for the revival and continued use of the therapeutic agent, light. It is considered that the benefit derived from pilgrimages made to shrines and to certain popular physicians of the times was due largely to the exposure of the pilgrims to the sunlight during their long journeys. Roy was one of the early popular healers who claimed to cure scrofula by laying his hands on those diseased. Many times the pilgrims did not reach him, but returned home improved or cured by the journey. Probably the first report of interest on this subject was presented by Faure before the Royal Academy of Paris in 1774. He dealt with the treatment of scars, wounds and tumors by sunlight. Lapeyre (1776) was successful in treating ulcers by the sun's rays concentrated with a lens. Two years later, Faure and Lecomte presented case reports of ulcers and cancer of the lip which they cured by heliotherapy. Bertrand, in 1799, contributed evidence as to the value of light in medicine. In 1815 Cauvin published a thesis "Des bienfaits de l'insolation" in which he advised parents to expose their scrofulous infants to sunlight. Diseases for which he recommends sunlight are scrofula, rickets, scurvy, rheumatism, paralysis, muscular weakness, and dropsy. He also reports a marvelous cure for a case of tuberculous rheumatism of Poncet.

Inspired by the work of Cauvin, Paulin Girard used heliotherapy for the treatment of chronic pulmonary catarrh, pulmonary tuberculosis, scurvy, dropsy, scrofula, and chlorosis. At the same time Monoyer was using sunlight for suppurative arthritis, peritoneal tuberculosis, and Pott's disease. About this time Grotthus formulated his law that only absorbed light rays are effective in producing chemical change. Richli was the first to commence the treatment of disease by irradiation in a sanatorium.

The discovery of ultra-violet rays by Herrschel placed heliotherapy on a scientific basis. In 1893 the important work of Finsen appeared. He called attention to the medical action of ultra-violet rays. The carbon arc lamp used by Finsen was improved by Kjeldsen. Wheatstone (1835) devised the mercury-vapor lamp as it is known now by passing an electric current through mercury-vapor. He noted that a bluish light was emitted which contained no orange or red rays. The first lamp which attracted public attention was perfected by Cooper-

Hewitt (1901). About this time Heraeus discovered that quartz crystals could be melted to make quartz receptacles. In 1904 Kromayer used water cooling attachments for the lamp. This and other suggestions of his applied to quartz lights have produced the present Kromayer lamp.

In 1890 Finsen first published his work on the use of ultra-violet rays in treating lupus. In 1902, Bernhard at Saint Moritz used sunlight to treat a suppurating wound of the abdomen. His method was based on his observation that Swiss butchers cured their meat in the sun. Bernhard soon published his technic for the treatment of surgical conditions by sunlight. Simultaneously, and unaware of Bernhard's discovery, Rollier at Leysin, in 1903, began his celebrated work. Among the important investigators using light therapy about the same time were Malgar (1904), Morin (1905), Escherich (1909), Bardenheuer (1911), and von Schrotter (1912). Morin was apparently the first to treat pulmonary tuberculosis in children with light.

Sunlight as Therapeutic Agent.—Palm (1890), as a result of a topographic study of the incidence of rickets, believed that sunlight should be regarded as a therapeutic agent. Buchholz (1904) published a paper on the light treatment of rickets and certain other diseases occurring in children. He used the "Glühlicht" (Kellogg), which, he stated, was poor in chemically active rays. Buchholz believed that the action of the lamp depended in part upon the heat rays and in part on the light rays. He treated 16 rachitic children with light and all showed general improvement. His work, however, did not attract the attention it deserved.

Neumann (1909) observed that rickets and tetany did not occur in Arosa, Switzerland (1740 meters), but occurred frequently at Davos at an altitude of 1560 meters. At Arosa the children lived much out-of-doors, whereas at Davos most of them lived under crowded city conditions.

Raczynski (1912) wrote: "It is the sun which plays the principal rôle in the etiology of rickets." He gave the first proof of the favorable influence of light on mineral metabolism by an experiment on puppies. One puppy was reared in the dark, the other in the sunlight, and both were suckled by the mother. At the end of 6 weeks the calcium and the phosphorus in the body of the puppy reared in darkness was very much less than that of the one reared in sunlight. He stated: "It is possible to assume that the lack of action of sunlight by influencing in so

unfavorable a manner the assimilation of calcium oxide in the young organism is one of the causes of rickets."

Ultra-Violet Rays and Rickets.—Huldschinsky (1919) reported that ultra-violet rays exerted a curative action on rickets. Winkler (1919) reported the favorable effects on the treatment of rickets with x-rays. Putzig (1920), Huldschinsky (1920), Riedel (1920), Erlacher (1921), Mengert (1921), and Hess and Unger (1921) extended these observations and established the therapeutic value of light. Sachs (1920-21) and Huldschinsky (1920) reported the cure of tetany with light treatment.

Hess and Unger (1920) reported the results of treatment of 5 rachitic children with the rays of the mercury vapor lamp over a period of 3 months. The entire body was exposed daily so that it became tanned. This treatment, they asserted, did not lead to a definite improvement in the rickets nor did it benefit their general condition. Violet rays, they stated, cannot be considered the equivalent of heliotherapy. They (1921) further studied the effects of ultra-violet and sun's rays and observed definite improvement and cure of infantile rickets.

Sunlight and Rickets.—Hess and Unger (1921) were the first to demonstrate by means of X-rays that sunlight alone possesses the same curative action as the light of the mercury vapor quartz lamp in human rickets. They exposed 5 infants with rickets to direct sunlight for periods of one-half to several hours daily. The general condition of the infants was benefited, as well as the condition of their bones. Hess and co-workers (1921) reported protection of rats against rickets by sunlight.

Shipley and co-workers (1921) reported the following experiment on the effect of sunlight on rickets in rats. Two groups of rats were fed diet 3143. One group was kept under laboratory conditions; a second group was exposed to sunlight an average of 4 hours daily for 62 to 67 days. The animals kept in the laboratory all showed gross and microscopic appearance of rickets; those exposed to sunlight showed no evidence of rickets. Although sunlight prevented rickets, the bones of these animals were undersized. Exposure to the sun's rays did not entirely compensate for the defects in the diet. The bones, though completely calcified, remained thin. They stated: "As the result of the gross and histological examinations made on the rats fed the rickets-producing diet 3143, but exposed to sunlight, it is possible to say that the changes produced by sunlight in the skeleton do not differ in any important respect from the changes produced

when the animals are kept in room light but on a diet supplemented by cod liver oil. Cod liver oil contains something which is essential for optimal cellular function. Light also contains something which is essential for optimal function. Cod liver oil or light, when made available to an organism previously deprived of either, permits the organism to put into successful operation adaptations or defense mechanisms which otherwise would have been ineffectual. Neither cod liver oil nor light meets the defects in the composition of the diet directly by supplying to the body either calcium or phosphorus, but meets them indirectly by so raising the potential of cellular activity as to secure the most efficient utilization possible of those substances available in the body which are directly or indirectly concerned with ossification and calcification."

Goldblatt and Soames (1923) placed two litters of rats on diets containing optimum amounts of calcium, phosphorus, and vitamin A. In the diet of one group vitamin A was supplied by cod liver oil, in the other it was supplied by butter fat. The rats receiving cod liver oil also had a liberal supply of vitamin D. Half of each litter was kept in the dark, and their litter mates kept in daylight, with an addition of daily irradiations for 20 minutes under the mercury vapor lamp. Since both groups were given an adequate diet, no difference was found between the two groups histologically or in the calcium content of their bones.

Sunlight and Anti-rachitic Value of Milk.—Luce (1924) says that the anti-rachitic value of milk depends upon whether or not the cow is kept in pasture or whether she is stall-fed in the dark. The vitamin D content of milk from stall-fed animals she found to be very inferior to that from cows kept in sunlight.

Boaz and Chick (1924) also discussed the influence of diet and management of the cow upon the deposition of calcium in rats receiving a daily ration of milk in their diet. They state that the influence of cod liver oil upon calcium deposition is not to be attributed to specific properties peculiar to that substance, but to a high content of the fat-soluble vitamin, since a similar deposition can be demonstrated in the case of cow's milk, the degree depending upon the diet of the cow and the amount of sunlight to which she is exposed.

Cod Liver Oil versus Ultra-Violet Rays in Treatment of Rickets.—Holmes and co-workers (1928) have compared the efficiency of cod liver oil with ultra-violet rays on young rats taking the same rickets-producing diet. As judged by growth,

roentgenograms, calcium and phosphorus of the blood serum and mineral content of bones, the mineral metabolism of the controls was decidedly inferior to that of the animals receiving the rachitic diet supplemented with ultra-violet light or cod liver oil. Aside from the control group, the average increase in body weight during the thirty-six day experimental period was greatest for the group fed 19.8 mg. of cod liver oil, and least for the group receiving direct irradiation and the group fed 14.8 mg. of cod liver oil.

Activation of Foods by Ultra-Violet Rays.—In June, 1924, Steenbock and Hess independently reported a remarkable series of experiments. Hess reported that cottonseed oil when irradiated with the mercury vapor quartz lamp at a distance of one foot acquired anti-rachitic potency as tested on rats. He further tested radium bromide, hydrogen peroxide and ozone, but found them without effect in rickets. He noted that irradiated mineral oil gave negative results.

Steenbock made the following claims in a patent application: By moderate (not over-) exposure to ultra-violet rays, the following become anti-rachitic: Foods, feeds, meat, cereals, grains, seeds, hulls, bran, oil, fats, corn oil, peanut oil, coconut oil, cottonseed oil, oleo oil, olive oil, lard, tallow, roughage, dog biscuit, chicken feed, butter, milk, baby foods, breakfast foods, the unsaponifiable lipoids of fats, brains, brain lipoid, cholesterol, oleomargarine, diet 3143, eggs, egg yolk. The activation may take place in carbon dioxide. The activating rays are filtered out by glass. The activated principle may be concentrated in the unsaponifiable fraction of fats, and the unsaponifiable fraction of fats is highly activatable. Injury results from prolonged irradiation. The light of the sun is unsatisfactory for activating. X-rays fail. The basis of the effect is suggested to consist in the emission of rays from the ingested material, but possibly it is due to a structural rearrangement of some constituent.

Steenbock and Black (1924) added the observation that rat rations irradiated by the mercury vapor quartz lamp can be activated to make them growth-promoting and bone-calcifying to the same degree as when the rats are irradiated directly. The activation takes place when the ration is irradiated in an open dish or in a stoppered Pyrex or quartz flask filled with air or carbon dioxide, but not in a brown bottle. The activation is not destroyed by subjecting the ration to a vacuum, heating it for 45 minutes at 96° C., or letting it stand for 24 hours at room

temperature. They suggested that "both light and the anti-rachitic vitamin may represent the same anti-rachitic agent—possibly a form of radiant energy."

They confirmed the observation of Goldblatt and Soames (1923) in finding that liver taken from irradiated rats is growth-promoting, whereas the liver from non-irradiated rats is inactive. The same was found true of the lung and muscle tissue. Inactive muscle, after removal from the body, exposed to the radiations of the lamp, was found to have become activated, being both growth-promoting and bone-calcifying. Liver treated the same way also promoted bone calcification. The activity of the liver taken from irradiated rats was not destroyed by drying at 96° C. for 24 hours and then keeping it in a stoppered bottle for 2 months.

Steenbock (1924) reported that alfalfa exposed to the sun in the field has feeble anti-rachitic properties. Hess and co-workers (1924) demonstrated that the active principle of irradiated linseed oil could be concentrated in the unsaponifiable residue. Hess and co-workers (1925) reported the activation of phytosterol from vegetable oils by ultra-violet rays. Lanolin after irradiation had little potency. Nelson and Steenbock (1925) found no evidence that irradiated air possessed growth-promoting or anti-rachitic properties as Hume and Smith had claimed. The effect was due to ingestion of irradiated feces.

Studies on Irradiated Cholesterol.—Drummond, Rosenheim and Coward (1925) reported that irradiated cholesterol induced resumption of growth in rats stunted on vitamin A deficient diets. It is now established that there is no relation between irradiated sterols and vitamin A. Hess and Weinstock (1925) confirmed the retention of potency of irradiated foods over a period of weeks. They traced the activatable principle to cholesterol and phytosterol, and found that activation takes place in nitrogen. They activated etiolated wheat seedlings and etiolated lettuce, but could not activate glycerol, gelatin or milk. It is now known that milk is activatable. Steenbock and co-workers (1925) could not activate purified protein, carbohydrates or fats, or salts, water, ether, hydrochinon, phloroglucin, or paraffin hydrocarbons. Cholesterol purified by crystallizations, then as benzoate, and finally as acetate as well as phytosterols, could be activated. Hess and co-workers (1925) suggested without data that skin is the acceptor of ultra-violet rays. Later they added dry milk, flour and spinach to the list of foods which they had

activated, and oleic acid and egg phosphatide to the non-activatable list. They also found activated cholesterol a preventive for rickets induced either by a low-phosphorus or a low-calcium type of diet, and that skin becomes active against rickets when irradiated. Saturated reduction products of cholesterol and phytosterol, terpines, cymene and citronella oil could not be activated. They reported (1925) that cholesterol undergoes a spectral change during irradiation. Activated cholesterol absorbs ultra-violet radiations to a less degree than ordinary cholesterol. If irradiated for many hours it becomes less transparent than non-irradiated cholesterol. Visible light, radiant heat and X-rays did not alter the absorption of cholesterol, and did not produce a spectral change in reduced sterols. The results recorded above were confirmed by Rosenheim and Webster (1925) for cholesterol, and using blood phosphorus as an index they found 0.2 mg. to be the minimum protective dose. They also suggested that a vitamin may be formed on irradiating the skin.

Hess and co-workers (1925) found that prolonged irradiation destroys the anti-rachitic properties of activated cholesterol; the process is not reversible. Activated cholesterol was found to keep best in oil, less well in water, and least well in a dry state. They found no difference in the oxygen content of non-irradiated cholesterol and the same after 1 hour of irradiation. Prolonged contact of activated cholesterol with acetone, chloroform or benzene did not cause a loss of activity. Bills (1925) found the anti-rachitic activity of cod liver oil and of irradiated cholesterol to be destroyed by n-butyl nitrite, which supports the view that the anti-rachitic principle in the two sources is the same substance.

Rosenheim and Webster (1926) found that after precipitating activated cholesterol by digitonin, 1/100 mg. per day of the dry substance not precipitated by digitonin prevented rickets in rats. They concluded that both the secondary alcohol group and the unsaturated carbon linkage of the sterol must remain intact for activation by ultra-violet rays.

Ergosterol—Provitamin D.—Windaus and Hess (1926) reported that if cholesterol is purified by the formation of derivatives (e.g., dibromide) or by treatment with mild oxidizing agents, it can no longer be activated by ultra-violet rays. They concluded that the mother substance of vitamin D is an impurity and not cholesterol. They stated that ergosterol may be the anti-rachitic provitamin, since it cannot be separated from cholesterol by physical methods, is very sensitive to oxidizing agents and is

active in preventing rickets in mere traces after irradiation. They pointed out that ergosterol presents an absorption band in the ultra-violet corresponding to that observed with active cholesterol.

Rosenheim and Webster (1927) purified cholesterol as far as possible by repeated crystallization, then converted it into the dibromide and regenerated the cholesterol by reduction. This purified cholesterol could not be activated by irradiation, which showed that it is not cholesterol, which is the precursor of vitamin D, but some impurity which is destroyed by bromination. The cholesterol thus obtained did not show the characteristic absorption bands of ordinary "pure" cholesterol. They announced that ergosterol, a sterol first isolated from oil of ergot by Tanret, is the parent substance of vitamin D.

Rosenheim and Webster (1928) present evidence that in ergosterol not only a typical ring structure but also the specific position of the three unsaturated bonds is essential for photochemical activation into vitamin D. According to Windaus and Linsert (1928), when ergosterol is irradiated it loses the property of being precipitated by digitonin. The number of double bonds is not changed during irradiation.

Bills and Brickwedde (1928) have activated ergosterol at the temperature of liquid air (—183°). They regard the process of activation as an isomerization involving rearrangement at a double bond.

Bills and Honeywell (1928) have described a procedure for isolating ergosterol of high purity from yeast. Ergosterol is the principal sterol of fungi, and yeast is the best source available. It contains two other sterols, zymosterol and cerevisterol. The ergosterol of yeast was shown to be identical with that from ergot.

Jendrassik and Keményffi (1928) state that irradiated can be distinguished from non-irradiated ergosterol by means of its color reactions with iodine.

Wave Length and Activity.—There is now general agreement that rays longer than 3,130 Å have little anti-rachitic value 313μμ). Protective action is exhibited by rays shorter than 313μμ. The shortest rays that reach the earth from the sun are 290μμ and, due to absorption by the atmosphere with its moisture, dust and smoke, and the varying distance of the sun from the earth, solar radiation often contains none shorter than 300μμ. A number of studies have been made with a view to discovering

glasses which are transparent to rays in the regions which are effective in the prevention or cure of rickets.

Hess and Weinstock (1923) state that the spectrum contains not only rays which can prevent or cure rickets but also rays which are able to neutralize or inhibit the effects of those which are beneficial. When the light of a mercury vapor lamp was filtered through glass of known transmission, they found that wave lengths above 302-313μμ had no curative action. They interpret their evidence to mean that the longer wave lengths (the visible rays of infra-red) inhibit the curative action of the utra-violet rays.

Hess and co-workers (1924) found that oils could be activated behind a Corning filter 586 A which cuts off rays at about 302μ. Steenbock and Black (1925) reported that the activating rays could not penetrate Corning glass filters G-385-DP, G-38-H, G-586-A, window glass, and plate glass. Water transmitted the active rays. Old oils, acid in reaction, could not be activated, nor could the unsaponifiable fraction of old oils, including phytosterol; but cholesterol from old acid oils, even after purification as benzoate and acetate was activatable.

Schlutz and Morse (1925), using recrystallized cholesterol, observed two absorption bands, the maxima of which were at 295 and 281μμ. These were undoubtedly the ergosterol bands accurately placed by Bills at 293.5 and 282. They observed that after brief exposure to ultra-violet rays these bands disappeared, which they interpreted to mean that the cholesterol had been at least half activated or that a small amount of some contaminant had been wholly changed.

Pohl (1926-27) and Morton, Heilbron and Kamm (1927) showed that when ergosterol is irradiated with ultra-violet rays three bands having maxima at 293.5, 282 and 270μμ disappear, while a new one at 247μμ becomes evident. Bills, Honeywell and Cox (1928) place the latter band at 248μμ, using highly purified ergosterol. They likewise studied the rate of activation of ergosterol during irradiation. The results are shown in the accompanying graph (Fig. 21). In this experiment the maximum potency was reached in 22.5 minutes, at which time the ergosterol was 250,000 as potent as the average cod liver oil. From this time further irradiation caused a falling off in potency, the product becoming inactive in about 3 hours. They show that oxidation plays no part in the activation of ergosterol, but is a factor in its destruction after activation. They suggest that the

absorption band at 248μμ is due to a substance having a molecular configuration similar to isoergosterol.

Irradiated 7½ Minutes.—The absorption curve closely resembles that of the non-irradiated solution, the maxima being only a little lower. *There is no evidence of any new absorption band,*

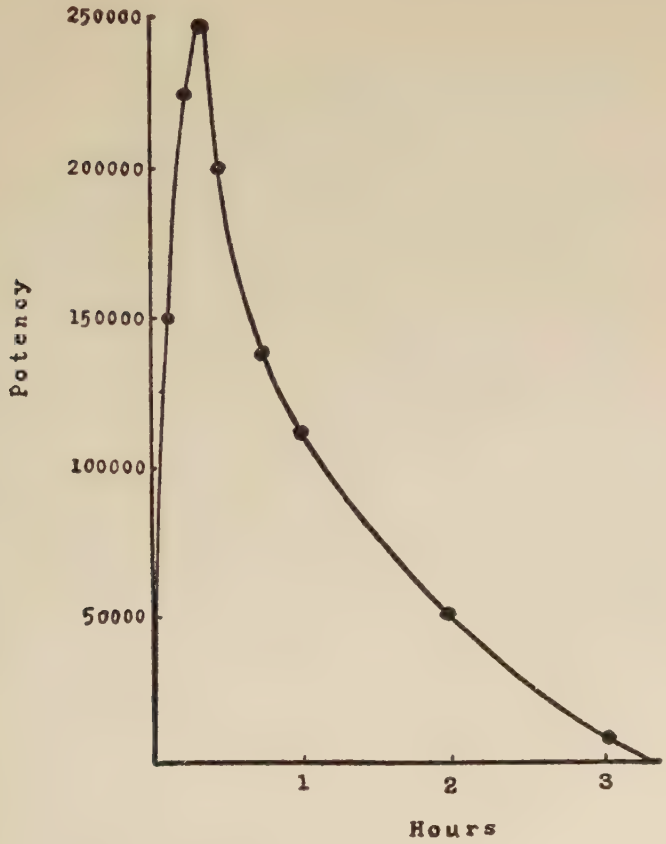


Fig. 21.—Effect of irradiation on the antirachitic potency of ergosterol.

yet the animal tests reveal the development of enormous antirachitic potency. The ergosterol has become 150,000 times as potent as average cod liver oil.

Irradiated 15 Minutes.—As yet there is only slight change in the absorption curve, and no evidence of a new band. The acti-

vation has nearly reached its maximum, the potency being 225,000 ×.

This figure shows that the potency of ergosterol is developed rapidly and reaches a maximum when about 73 per cent. of the ergosterol is still present. The decline of the curve is the result of destruction of activated ergosterol and reduction of reserve inactive ergosterol to a point where destruction overtakes the formation.

Effects of Infra-Red Radiation.—A remarkable observation on the effect of infra-red radiation has been reported by Clausen (1928). Ten minutes daily exposure to near infra-red radiation (ranging from 720-1120μμ) of an intensity 0.132 gm. cal/min/cm^2, will produce a definite effect upon young rats fed upon a rickets-producing diet. This effect is not fully apparent until the fifth or sixth week of experiment. The radiation causes increased growth of the animals, and prolongs by a period of 4 to 6 weeks their survival on the rickets-producing diet, without conferring any protection against the disease. Infra-red radiation, therefore, acts in a manner different from ultra-violet, and exerts a physiological action whose effect can be demonstrated.

In a limited number of animals examined, this growth-promoting effect of infra-red radiation was found to be associated with marked enlargement and hypertrophy of the thyroid gland. Irradiation with ultra-violet, in corresponding littermates, was found to prevent this hypertrophy whether or not the rat was exposed to infra-red radiation. This result must be corroborated by further experiment.

Some hyperplasia of the parathyroid gland was observed in a group of rats receiving infra-red radiation. The glands of the corresponding group, receiving ultra-violet, were of normal size.

Ash analysis of the bones of 18 rats protected against rickets with ultra-violet radiation showed slightly higher ratios between the ash and organic residue, than those of a similar group of litter mates that had received in addition to the ultra-violet, a daily exposure to near infra-red radiation.

Therapeutic Application of Activated Ergosterol.—The researches on ergosterol constitute a great triumph, and vitamin D preparations derived from ergosterol by the action of ultra-violet rays may in the future replace cod liver oil as a therapeutic agent. All clinical experience with irradiated ergosterol as a substitute for cod liver oil supports this view.

Some pediatricians have expressed alarm over the possibility

that too much vitamin D may be taken by people as the result of the availability of active ergosterol in proprietary preparations and of inactive ergosterol in yeast, which is now freely eaten by many persons in the belief that it is a health-promoting food. The high intake of ergosterol which would result from eating liberal amounts of yeast would presumably increase its concentration in the skin; and if now the skin be exposed to sunlight or to ultra-violet rays, a practice which has found great favor with the public of to-day, an excessive amount of ergosterol might be activated in the skin, thus producing an excessive amount of vitamin D.

Pfannensteil (1927) states that small doses of vitamin D raise the bactericidal power of the blood, whereas large doses caused severe intoxication and death. Kreitmair and Mall (1928) stated that a daily dose of 20 mg. of irradiated ergosterol caused the death of white mice in 6 days. A daily dose of 1 mg. was fatal when continued for 20 days, and large doses caused death more rapidly. Five mg. per kilogram of body weight daily proved fatal to rabbits, cats, and dogs. Post-mortem examinations showed extraordinary deposits of calcium in blood-vessel walls, heart, stomach, lungs, kidneys, and muscles. Changes were noted in the spleen. The results suggest the sclerosis induced in rabbits by feeding cholesterol over long periods, and similar results have been obtained by feeding ergosterol. The changes resulting from feeding ergosterol 2 to 3 months were said to be induced in 8 to 16 days by irradiated ergosterol. It should be pointed out that the amounts of activated ergosterol fed in these experiments were vastly larger (about 1000 times) than the amount necessary for the prevention or cure of rickets. On the same basis 100 mg. daily would be necessary to produce unfavorable effects in man, whereas only 5 mg. daily have been advised for the cure of rickets, tetany, or osteomalacia.

Harris and Moore (1928), of the Medical Research Council of Great Britain, made an inquiry into the effect of feeding excessive amounts of vitamin D. Young rats given synthetic diets containing 0.1 per cent. of irradiated ergosterol (i.e., 100,000 times the concentration adequate for protection from rickets) invariably lost weight rapidly and died. Non-irradiated ergosterol given to controls in the same amount caused no loss of appetite or deviation from the normal growth rate. Feeding 0-0.001 per cent. of irradiated ergosterol produced no ill effects. They point out that when large doses of activated ergosterol are adminis-

tered it is conceivable that the injurious effects are not due to the irradiated ergosterol but to impurities.

Dr. C. E. Bills informs the writers (personal communication) that he has kept rats through half the span of life, feeding them daily 100 times the curative dose of activated ergosterol without noticeably ill effect. From these results no apprehension need prevail concerning the danger of applying activated ergosterol as a therapeutic agent. Among the drugs commonly used in medicine few which are of extraordinary value when administered in proper doses would not produce profound injury if given in 100-1000 times the recognized beneficial doses. Ergosterol is, however, a substance with marked pharmacological properties and should be used only with knowledge and discretion by the medical profession.

Hess and Lewis (1928) state that irradiated ergosterol is by far the most potent of the anti-rachitic agents, and that it is highly specific. They state that cod liver oil in the amount in which it can be given is a specific of limited dependability, only moderately effective for the average infant, uncertain in action for the rapidly growing infant, and ineffective for the premature. Irradiated ergosterol is quite as valuable in tetany as in rickets, and in both disorders is remarkable for the rapidity, as well as for the reliability of its action. Their studies show a favorable influence upon the regulatory action of activated ergosterol on both blood calcium and phosphorus. No elaborate study has yet been made to determine the proper dosage.

Sources of Ultra-Violet Rays.—The percentage of ultra-violet radiations present in sunlight is exceedingly variable, depending on atmospheric conditions, especially the moisture present in the air. Hence it becomes necessary to obtain ultra-violet radiation artificially by one of the following means:

1. Chemical lamps which burn certain fluids in oxygen. These are seldom used, as they are difficult to operate and possess a limited field of radiation.

2. High tension disruptive elective sparks discharged between metal electrodes as, for example, tungsten.

3. Carbon arcs.

4. Mercury vapor arcs in vacuo.

The three latter sources have found application in medicine. The lamp adopted by Finsen contained a carbon arc light of high candle power. The light was concentrated by a series of quartz lenses which were water-cooled. Finsen's aim was to obtain a

light with the maximum ultra-violet rays and a minimum of heat rays which cause burning. Around each light were hung four concentrating telescopes connected to an iron ring suspended from the ceiling. Although the light was cooled, it was too hot to apply directly; hence Finsen devised a small apparatus consisting of a brass ring enclosing quartz lenses. This is water-cooled. The apparatus is pressed against the skin to be treated; it cools the surface and expels the blood from the skin.

Many mercury arc lamps have been invented and modified since 1835, when Wheatstone first observed that a brilliant bluish light is emitted when mercury is vaporized in an electric arc. The mercury arc produces a light rich in the shorter ultra-violet rays. Kromayer devised a water-cooled mercury vapor quartz lamp which is used for local applications. The Burdick water-cooled lamp is similar to this. The air-cooled lamps are employed for general radiation.

Ultra-Violet Rays and Resistance to Infection.—Pugh (1925-26) studied the effect of artificial light treatment on children's power to resist infection. He found no evidence of increased resistance (e.g., epidemic catarrh, chicken pox) among children given artificial light treatment. It may be, however, that his patients were already receiving optimum exposure to the light of the sun.

Barenberg, Friedman and Green (1926) report on the effect of ultra-violet irradiation on the health of a group of infants in an orphanage. Irradiation with the mercury vapor quartz lamp was found to be associated with an initial increase in weight, but this increase did not continue during the subsequent months. Irradiation did not lessen the number of infections during the winter, whereas during the spring there was a definite decrease. Ultra-violet rays did not protect against pertussis. The hemoglobin was not increased, but its percentage rose with the advent of spring and the diminution of infection. Irradiation improved the texture of the skin and the turgor of the muscles. The pigmentation brought about by the ultra-violet rays differed somewhat in hue from that which follows heliotherapy.

MacKay (1927) discusses artificial light therapy in infancy. She compared 66 cases treated with mercury lamp with 137 control cases. Observations over 13 months were made on the effects of treatment with a mercury lamp on the health of out-patient infants artificially fed. For the most part they came from poor and overcrowded districts in the East End of London,

and were subnormal in general health and weight. The rate of gain in weight of controls was approximately that of the light cases, whether the light group is considered as a whole or comparison is made only with a section of the cases having more intensive treatment. The differences observed were under 2 per cent. of the normal weight and these are probably due to chance. The hemoglobin in the blood was approximately the same in the controls and in the light group. Light treatment did not cure or prevent the development of anemia. A satisfactory comparison of resistance to infection is difficult, but the amount of intercurrent illness in the light group and controls appeared about the same in winter. In summer the figures suggest that the controls showed fewer respiratory infections. The author's notes made at the time suggest that in the late winter and spring months the light cases, as judged by color, general nutrition and appearance of health, seem to have made greater progress than the controls. But no objective evidence was obtained to support these findings.

It should be remembered that the negative results obtained from this investigation cannot be interpreted as necessarily in opposition to any positive findings obtained by others with similar cases; since the dosage of artificial light is entirely empirical, different results might have been obtained with another type of lamp, e.g., the carbon arc, and had the exposure to daylight been less for all cases the contrast in light exposure between the two groups would have been greater.

From the above records it is evident that beneficial effects of irradiation are to be expected only in certain definite conditions and that there is now a tendency to over-exploitation of lamps for the treatment of many conditions where it is of little or no value.

The carbon arc light is the best substitute for sunlight. The open carbon arc is used considerably in lamps abroad, where it is marketed as the aureol lamp. It is much cheaper than the quartz mercury-vapor type. Lamps with tungsten, iron, zinc, nickel, magnesium and other metals to form the arch have been used. Lamps which furnish only red rays have also been marketed, but research with these has not proved satisfactory.

Polarized Light and Avitaminosis.—Macht and Stepp (1926) studied the effect of polarized light in experimental avitaminosis. In animals with manifestations of dietary deficiency already developed, polarized light apparently exerts a profound effect

inasmuch as a considerable number of the animals died during the irradiation. Frequently tonic-clonic convulsions occurred prior to death. The effect of the light was particularly marked in animals with a combination of disturbances such as xerophthalmia and rickets.

Sunshine, Skyshine and Rickets.—Since of all the known vitamins none is so poorly distributed among foods as the anti-rachitic factor, and since it appears that in many vegetable oils there occur sterols which can be activated to form vitamin D by ultra-violet rays, the effectiveness of available sunshine becomes a matter of great importance. Short ultra-violet rays are readily cut off by the smoke, dust and moisture in the atmosphere. They are therefore markedly reduced in winter and in the early morning and late afternoon, when the rays of the sun pass through a greater distance of atmosphere than when the sun is high in the sky.

The observations of Tisdall and Brown (1928) made in Toronto, may be taken as representative of sunshine studies of the temperate regions. The average number of hours of sunshine there each day for three years was 1.9 for December, the lowest figure, to 9.5 in July, the highest. The average percentage of maximum sunshine from 11 A.M. to 1 P.M.—the period during which infants can be most conveniently exposed—showed a similar increase from December to July. The sun's rays during December, January and February produced a slight but definite anti-rachitic effect on rats. A sharp increase occurred in the anti-rachitic effect of sunshine about March 1. The anti-rachitic effect in April and May was approximately 8 times as great as in December, January, and February. This is believed to be due not to an increase in the amount of ultra-violet rays so much as to the presence of rays that are shorter than those found during the winter. The anti-rachitic effect of skyshine (rays reflected from sky and clouds) is approximately one-half to two-thirds as great as that ordinarily termed sunshine, which consists of rays from the sun plus the reflected rays from the sky.

Clark (1928) states that in Baltimore during March, April and May, 1928, the intensity of ultra-violet rays increased from March to May, but that it was necessary to expose for 15 hours at 5 meters from a north window of ultra-violet transmitting glass to get as much ultra-violet radiation as can be obtained by 2 minutes out-of-doors in the direct sun at noon. She states that any child going out-of-doors for recess or when going out

to lunch will get more ultra-violet radiation than would be secured all day behind a window of ultra-violet transmitting glass. She suggests the usefulness of these glasses in solariums, in animal houses and zoos, but discourages their installation in school buildings.

Eddy (1928) compared a series of window glasses. He kept little rats on a rickets-producing diet exposed to sunlight in New York City, the light passing through glass before falling upon

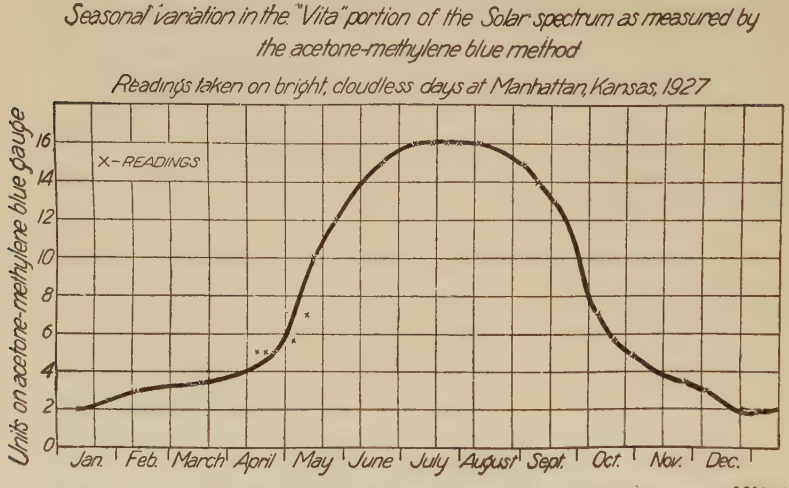


FIG. 22.—(Courtesy of Professor P. S. Hughes, Kansas State Agricultural College.)

the animals. His tests warrant the assumption that schoolrooms equipped with Vitaglass would admit enough ultra-violet rays in winter months to heal or prevent rickets if the weather is clear and the exposure sufficiently long. Only that part of the room directly in the path of the rays receives benefit. He suggests that it is probably wiser to provide solaria in which children can be exposed without clothing to direct sunlight without exposure to cold.

The several studies reported show clearly that it is far more important that people spend a certain amount of time daily in the sunlight out of doors, than to seek to secure active rays through any kind of windows available. For persons too ill to be taken out, treatment with artificial sources of ultra-violet rays will be more effective than sun's rays which have passed through any kind of glass now available, or even than quartz

panes, which are too expensive for general use. It appears to be established by sound observations that the beneficial effects of ultra-violet rays are limited to a few well-defined conditions such as surgical tuberculosis, rickets, and osteomalacia. At present there is a tendency to exaggerate the claims as to what can be accomplished by ultra-violet ray treatment. It is so easy to do harm by excessive irradiation that ultra-violet lamps should be used only on the advice and under the supervision of a well-qualified physician.

CHAPTER XXI

CALCIUM ASSIMILATION IN FARM ANIMALS

Green Food versus Dry Food and Calcium Assimilation.—Steenbock and Hart (1913) presented data which showed that there were marked differences in the amount of calcium eliminated in the feces of a milking goat when the animal was changed from old, dried roughage to green pasture. During the earlier part of the experiment when the animal was on dry feed there was progressive increase in the content of calcium in the feces. When the goat became debilitated, it was placed on June grass pasture, and was given oats in addition; it rapidly recovered with increase in weight. After 2 weeks it was returned to the metabolism cage for a new calcium balance record. After the period of green feeding the fecal calcium elimination was so reduced that the animal gave a positive balance as compared with a high fecal calcium output, and negative calcium balance on the dry ration. The interval of green feeding therefore enabled the animal to again assimilate the calcium of the dried feeds, which it had become unable to do after confinement to the dry feed for a considerable interval.

Forbes and associates (1916-18) have made many important contributions to the subject of calcium metabolism by showing that high milking cows receiving rations that were supposed to provide an ample intake of calcium may, nevertheless, eliminate a larger amount of calcium than was ingested. Addition of calcium salts to a ration of grains, dry alfalfa hay and corn silage, did not establish positive calcium balances.

Meigs, Blatherwick and Cary (1919) extended these studies by showing that a dry but pregnant cow probably does not assimilate sufficient calcium from a calcium-rich ration such as dry alfalfa hay, corn silage and a grain mixture to keep her in positive balance, but transfers calcium salts from her skeleton for fetal skeleton building.

Hart and co-workers (1920) reported experiments with cows and goats which showed that milking animals receiving grains

and dry oat straw are brought into a decidedly negative calcium balance. There was a larger fecal calcium, indicating a failure of calcium assimilation. With milking goats fed a dry ration there was a negative balance of 1.6 to 2.3 gm. of calcium oxide per day, but when the dry straw was replaced by an equivalent amount of fresh green material the negative balance was 0.6 gm. per day.

Hart, Steenbock and Hoppert (1921) found that oat hay dried out of direct sunlight, but in a fairly well-lighted attic, seemed to retain the property of the fresh green oats. One hundred and twenty to 240 c.c. of orange juice per day did not improve calcium assimilation. Raw cabbage was not superior to dried cabbage, so vitamin C was eliminated as a factor in calcium assimilation. Five to 10 c.c. of cod liver oil changed a negative to a positive calcium balance.

Hart, Steenbock, Hoppert and Humphrey (1922) found that liberally-milking cows were maintained in positive calcium balance when 10 lbs. of dry alfalfa which had been cured under caps was used as a roughage and as the main source of calcium, but supplemented with corn silage and a grain mixture. They had formerly not had a calcium balance with dry alfalfa fed to dairy cows, and state their belief that possibly there is a variation in the kinds of alfalfa. Forbes did not secure calcium retention with dry alfalfa as the principal source of this element. Hart and co-workers also found that with fresh green alfalfa there was a more effective storage of calcium than when the animals were on the dry plant. They noted a positive phosphorus balance when the animals were in a positive calcium balance. Hart, Steenbock and Bethke (1922) did not bring about calcium and phosphorus equilibrium in milking cows by feeding alfalfa which was cured in a window exposed to the air and light for 4 days; in drying, a difference in the destruction of the vitamin assisting in calcium assimilation apparently took place. Supplementing timothy hay with bone meal did not result in establishing positive balances.

Telfer and Crighton (1924) found from a study of normal and modified goat's milk fed to kids from birth that the normal mineral content of milk is probably near the minimum requirement of the species. The rate of ossification could be considerably increased by a greater concentration of mineral matter; a small reduction in mineral matter is followed by defective growth and impaired nutrition.

Meigs and co-workers (1925) found changes in the calcium and phosphorus content of the bodies of milking cows not to run exactly parallel, but a long-continued loss of calcium from the body will cause a corresponding loss of phosphorus even though the ration contain sufficient assimilable phosphorus. The rate

FIG. 23.—Pigs fed the basal ration of white corn, wheat middlings, linseed meal and NaCl. (From Ohio Experiment Station Bulletin 395, courtesy of Dr. G. Bohstedt.)

of calcium assimilation from hay depends on its treatment after being cut. The rate was very low for hay that had been exposed to rain. From well-cured timothy and alfalfa hay, about 20 per cent. of the calcium intake was assimilated.

FIG. 24.—Pigs fed basal ration of white corn, wheat middlings, linseed meal, NaCl and 5 per cent. alfalfa meal. (From Ohio Experiment Station Bulletin 395, courtesy of Dr .G. Bohstedt.)

Steenbock and co-workers (1925) also found a remarkable influence of exposure to active light rays on the anti-rachitic properties of hays. Clover hay exposed to sunlight had considerable calcifying power, while that made in the dark was inactive. Excessively weathered hay was reduced in anti-rachitic action

but its value was markedly increased by exposure to a mercury vapor lamp.

Hart and co-workers (1926) found that cows producing 40 to 60 lbs. of milk daily were in negative calcium balance on a ration of green supplements, corn silage and green grass, when they were kept out of direct sunlight. On the same ration with the cows exposed to direct sunlight in June, negative calcium balance was still continued but at a reduced rate. Sunlight plus a ration with the calcium content comparable to the average summer ration fed dairy cows in summer time did not maintain

FIG. 25.—From lot of pigs fed basal ration of white corn, wheat middlings, linseed meal and NaCl. (From Ohio Experiment Station Bulletin 395, courtesy of Dr. G. Bohstedt.)

calcium equilibrium. Summer sunlight in comparison with irradiation of a mercury lamp is feeble in its anti-rachitic properties when considered in relation to liberally milking animals.

They further observed (1927) that the non-saponifiable fraction of cod liver oil fed to lactating goats is not effective in improving calcium assimilation, but marked improvement resulted when the pasty mass was dissolved in corn oil, which oil itself has no effect on calcium assimilation. Further studies on calcium assimilation as affected by green feed, sunlight and calcium additions are recorded by Hart, Steenbock and Scott (1927).

Osteomalacia in Animals.—Van Sageghen (1919), in discussing osteomalacia of horses, speaks of it as osteoporosis. Whenever the disease occurred among horses or donkeys in the Belgian Congo, the soil of the district was found to be very poor in calcium and phosphorus, and little or no calcium was found in the

water. No cases occurred in districts in which the water contained calcium.

Stammers (1924) found that after prolonged drought South African cattle frequently show a chronic edema. In edematous cattle, bones showed a marked increase in moisture and a deficiency in protein and in fat. The ash content diminished and there was a marked decrease in phosphorus content. The adipose tissue was largely replaced by moisture and the bone marrow showed diminution of fat. The marrow was gelatinous, a condition seen in hunger and certain pathological conditions. Most South African soils are deficient in phosphorus. Crawford (1927) has described skeletal defects of herbivora in Ceylon, and Delft Island.

Palmer and Eccles (1927) found that numerous cattle in Minnesota suffer from a mineral deficiency which causes stunting, osteophagia, general pica, delayed oestrus, and other metabolic disturbances. The phosphorus content of the hay was often below 0.2 per cent. Administration of phosphate cured the condition. Maynard (1926) concludes that in the feeding of farm animals, sodium chloride and a salt of iodine should always be added, and that the calcium and phosphorus contents are in general insufficient. He found no evidence that magnesium, potassium, iron or sulphur additions to farm rations are necessary. Reproductive disturbances are less frequent when sufficient calcium and phosphorus are provided. He also states it is not proven that there is an optimum vitamin content in farm rations generally. Cod liver oil is advantageous to milking animals.

Rickets in Swine.—Bohstedt and co-workers (1924) discuss the problem of rickets in swine as affected by nutrition. They state that lameness, unthriftiness, partial paralysis, or rickets in pigs is very prevalent, especially in winter and early spring. These abnormalities in a pig may be symptoms of one and the same disorder. Grains or seeds and their by-products used so generally in winter rations of hogs, furnish neither enough nor the right kinds of minerals and vitamins. It is apparently due to these two deficiencies that rickets occurs. Addition of calcium carbonate, phosphate as di-sodium phosphate or bone flour, without the addition of vitamins, did not prevent rickets. The bones of pigs fed a diet very low in minerals, but containing cod liver oil, could be easily broken by a twist of the hand. Many spontaneous fractures occurred in pigs fed the basal ration and cod

liver oil alone. Feeding both cod liver oil and bone flour produced the most uniform and economical gains.

Leg Weakness in Pigs.—Maynard (1922) reported studies on leg weakness or posterior paralysis in pigs. Four pigs at the age of 12 weeks were put in a dry lot and were fed for 90 days the following diet: 1 part yellow hominy; 1.4 parts of pasteurized skim milk, together with a salt mixture of charcoal, ground limestone, and sodium chloride. After 90 days 3 of them had developed stiffness of the hind legs and were in a weakened condition. The skin was dry and scaly, and contained livid spots. One pig had a swollen knee. The stiffness increased and was accompanied by great pain. Two of the animals developed paralysis in the hind legs and could not stand. There was rapid loss of weight. The mineral mixture was then replaced by precipitated bone meal, precipitated calcium carbonate and sodium chloride, and four ounces of raw carrots were given daily. At the end of 5 weeks the stiffness had disappeared and the skin was nearly normal. They had completely recovered at the end of 7 weeks. Possibly incipient scurvy may have modified the bones of these pigs, and if so, the carrots played a special part in inducing their recovery.

Maynard and co-workers (1923) state that the essential lesions in the posterior paralysis of pigs are an osteoid rather than an osseous tissue formation at the epiphyses and costo-chondral junctions. These changes indicate a diminished calcification somewhat suggestive of rickets, but without the usual deformities found in this disease. The condition is not due to a deficiency of calcium and phosphorus in the diet, since these were supplied. It seems to be due to a lessened assimilation of these materials. Addition of either alfalfa or cod liver oil produces recovery of the cases already affected. The symptoms in the early stages and the lesions in the parenchymatous organs are similar to those that are supposed to be diagnostic of hog cholera.

Calcium Metabolism of Laying Hen.—Wheeler (1919) found that rations deficient in calcium but carrying an abundance of magnesium produced a noticeable shortage of calcium and of mineral matter in the bones of fowls and ducks. In no instance was magnesium found to serve to any extent in place of calcium for such purposes as egg shell material, when calcium was lacking. Strontium was capable of replacing calcium to a considerable extent in the egg shell and in the bones. Fewer eggs were

obtained from hens fed added magnesium or strontium salts than those fed calcium salts.

Buckner, Martin and Peter (1924) reported experiments on the calcium metabolism of the laying hen. They found that hens receiving limestone produced larger eggs than those which did not receive it. The hens receiving corn, buttermilk and limestone produced larger eggs than those receiving corn and a mash containing tankage and limestone, and both the shell and liquid part were heavier. The addition of calcium carbonate greatly increased the efficiency of buttermilk for egg production. The hens on a diet of buttermilk and corn were at all times in a notably better condition than those fed corn and a mash containing tankage but no buttermilk. The practical conclusion for the poultryman is that for satisfactory egg production, laying hens on the above diets must be given additional calcium, which should be in the form of the carbonate. They (1925) fed hens a diet of corn, wheat, and buttermilk, with additions of green food twice a week, and secured eggs with lighter shells than with the same ration plus ground oyster shells. The embryos in the low-calcium eggs seemed to lack vigor, and many died during incubation. Later (1926) they found that the addition of calcium carbonate to the feed increased the weight of the eggs, shells, contents, and dry weight of the chicks hatched. The quantity and percentage of phosphorus are the same in strong and weak chicks, but the quantity and percentage of calcium are less in weak chicks.

Parhon (1926) found the serum calcium in hens increased during laying and diminished during the setting period. He attributes the differences in the calcium content of the serum to the differences in the psychic states of the fowls during these periods.

Bethke, Kennard and Sassaman (1927) found yolks of eggs from hens having access to blue grass approximately 5 times as potent in A and 10 times as potent in D as yolks from control hens.

Holmes, Doolittle and Moore (1927) studied the stimulation of reproduction in pullets by the fat-soluble vitamins. Tests were made over 32 weeks with oil having about 1660 units per gram, the birds being fed 0.25, 0.5, 1.0 and 2.0 c.c. of oil. Egg production increased, weight of eggs slightly increased, and the number of blood clots diminished as the oil was increased. The hatch increased, viability of chicks increased, but broodiness was not influenced. The body weight of the high producing birds was

not decreased, and the mortality among the high test birds was decreased over the controls. The eggs and flesh contained no detectable flavor. The vitamin potency of eggs increased over the control birds.

Effect of Sunlight on Development of Chicks.—Hart, Steenbock, Lepkovsky and Halpin (1923) studied the effect of exposure to direct sunlight on the growth of chicks. They found that one-half hour's exposure to direct sunlight was more potent in furnishing the anti-rachitic equivalent than was 5 per cent. of the synthetic ration fed as green clover calculated on a dry basis. They fed two groups of chicks on the following diet: Corn 97.0, NaCl 1.0 $CaCO_3$ 2.0 per cent., and skim milk ad libitum. One group was fed white corn, the other group yellow corn. Both groups were exposed to sunlight daily. The birds receiving the white corn, which was low in vitamin A, did not respond to the sunlight treatment, whereas those receiving yellow corn and sunlight grew to be beautiful birds.

Hughes and Payne (1924) studied the effect of exposing pullets ten minutes per day to a source of ultra-violet light. During 16 weeks 12 irradiated hens laid 497 eggs as compared with 124 eggs by the control flock. The eggs from the irradiated hens had 30 per cent. more calcium in the shell and 5 per cent. more calcium in the contents than did the control eggs. The average hatch of eggs from the treated hens was 76 per cent. against 40 per cent. for the controls. The controls began to show leg weakness and retention of eggs by the sixteenth week, and several died. After a rest period of 19 days, the irradiated hens were deprived of ultra-violet light, while those in the original control group were irradiated; this led to a complete reversal of the previous record.

Hart and co-workers (1925) found egg production by hens is greatly influenced by the supply in the diet of vitamin D and by the effects of light containing ultra-violet rays, even when the ration furnished an abundance of calcium. Hens continued to lay abundantly on a ration when given exposure to ultra-violet rays for 10 minutes daily, whereas those not receiving the light decreased their egg production practically to the zero point. Feeding cod liver oil, or irradiating hens after long confinement to a rachitic diet, with resultant decrease in egg production, again stimulates and increases egg production. The hatch of eggs from irradiated hens was sustained at 60 to 70 per cent., while in its absence the hatch receded to zero. Irradiation

increased markedly the amount of lime in the shell, whereas the calcium and phosphorus content of whites and yolks was not affected. Embryos of eggs from irradiated hens contained, after 21 days of incubation, nearly twice as much lime as the embryos from non-irradiated hens' eggs. The lower hatch of eggs produced from a rickets-producing ration is believed to be related to the lower power of the developing embryo to transfer lime from the shell. The vitamin D content of yolks from irradiated hens was about ten times that of yolks from non-irradiated hens. Fertility of eggs was not consistently influenced by a paucity or abundance of the anti-rachitic factor.

Hughes and co-workers (1925) found that the amount of ultra-violet radiation which a hen receives is an important factor in determining the vitamin D content of eggs produced when the feed is low in this principle. Thirty minutes of ultra-violet treatment daily is insufficient to enable hens to store sufficient D in their eggs to entirely prevent rickets in chicks fed rations containing these eggs, although the severity of the rickets is materially reduced. Eggs from hens receiving sunlight through glass contain practically no anti-rachitic value. Eggs low in vitamin D do not hatch so well as those with a high D content, other factors being constant.

Hughes, Titus and Moore (1925) treated 4 groups of hens with different amounts of ultra-violet rays, and the eggs of each lot were fed to growing chicks. The chicks which received eggs from hens receiving direct sunshine, developed normally, as shown by appearance, and analysis of blood and bones. Chicks fed eggs from hens that received sunlight filtered through glass plus light for 30 minutes per day from a mercury vapor lamp developed some rickets, but they were in better condition than chicks fed eggs from hens which received no ultra-violet rays. Eggs from hens receiving the least ultra-violet rays were poorest in quality.

Price (1926) states that a sufficiency of calcium and phosphorus in the diet is essential to health and growth of chicks in order that they may derive benefit from food exposed to the sun's rays or from vitamin D in the diet. Exposure of cod liver oil to bright sunlight for a short time increases its effect on calcium metabolism, but prolonged exposure is deleterious, and in some cases results in the formation of poisonous substances.

Nonidez and Goodale (1927) showed that the combined effects of lack of sunlight and a ration poor in vitamin C led to the

enlargement of the parathyroids in growing chicks. There was an increase in cell size and in the number of epithelial cells. This was followed by a phase of regression and in some cases by local degenerative changes. Subsequent exposure to direct sunlight resulted in decrease of gland volume with considerable crowding of epithelial cells and sometimes pressure atrophy.

Rickets in Chicks.—Hughes (1924) found a condition to develop in chicks identical with rickets in mammals when the birds were given satisfactory food, but received sunlight filtered through glass. Chicks receiving the same food and treatment, but exposed to direct sunlight for a few hours daily, developed normally. Ultra-violet light had the same beneficial effect as direct sunlight. He also (1924) found that 0.5 per cent. of cod liver oil was effective in preventing leg weakness, but 10 per cent. of either sprouted oats or green alfalfa was not sufficient to prevent the condition. Ultra-violet light had a marked beneficial effect on the well-being of the chicks, as shown by an increase in the number of eggs produced, their chemical composition, and the hatch.

Dunn (1924) reported experiments on chicks which indicated that cod liver oil which had been preserved in a dry mixture containing 85 per cent. of starch and 15 per cent. of oil, which was corked in bottles and stored in the dark for 6 months, had lost its anti-rachitic properties as compared with raw cod liver oil which had been stored for the same time and under the same conditions. Hart, Steenbock and Lepkovsky (1925) found, on the other hand, that cod liver oil, mixed with ground grains and stored in cans at room temperature, retained its calcifying power for at least 6 months, as shown by tests on chicks.

Murray, Little and Bovie (1924) found that chicks exposed only for a few minutes each day to the rays of a mercury vapor arc showed more rapid bone formation than did chicks which were exposed many hours each day to direct sunlight. Failure in growth of chicks receiving an insufficient amount of ultra-violet light was traced to a failure in the development of bone tissues, even though the chicks were provided with a sufficient amount of calcium and phosphorus. Without light, these salts did not deposit in the bone.

Leg Weakness in Chicks.—Hart, Halpin and Steenbock (1921) reported that green cabbage, green clover or orange juice when fed with a synthetic diet in the absence of sufficient roughage, did not prevent leg weakness in chickens. Ten per cent. of

paper was a very good roughage for chicks; with the use of paper and a synthetic diet chicks made good growth. They determined that leg weakness is not scurvy in chickens. Young chicks were

FIG. 26.—A contrast between the effect of green plant tissue and radiant energy. Both chicks received a synthetic ration composed of alcohol-extracted casein 18, dextrin 60, salt mixture 5, agar-agar 1, yeast 15, and 5 per cent of fresh green clover calculated on the basis of the dry weight of the clover.

	Left	Right
Initial weight, June 19, 1923	35 grm.	38 grm.
Weight, July 10, 1923	77 grm.	95 grm.

July 12, 1923, began exposure of one chick (right), to sunlight one-half hour daily, both birds being continued on the same ration.

	Left	Right
Weight, August 8, 1923	95 grm.	235 grm.

(Courtesy of Professor E. B. Hart, Wis. Agr. Exp. Sta.)

reared under confinement to 800 gm. or more in weight on a diet of white corn, skim milk, a salt mixture, and cod liver oil (1922). Wood shavings were provided for litter. The experiment was continued during 11 weeks, which they state is beyond the

critical time for the development of leg weakness. Chicks require a liberal supply of the vitamins in cod liver oil during the growing period. Vitamins B and C do not appear to be so necessary, although under certain conditions the requirements for vitamin B are high. When cod liver oil was omitted, the birds died in from 4 to 6 weeks with symptoms of leg weakness. The inorganic phosphorus content of the blood was low in these birds as compared with those receiving cod liver oil.

Mussehl and Calvin (1921) presented data which show that the application of the biological method for the analysis of a

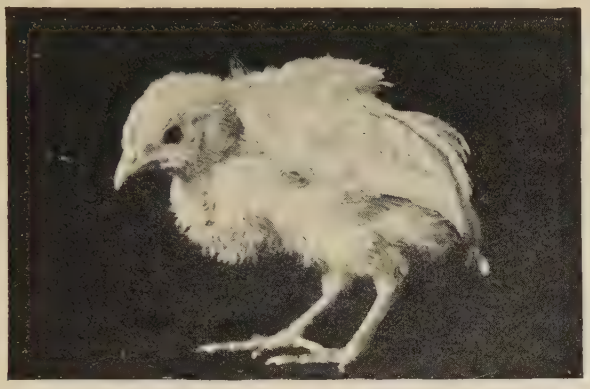


FIG. 27.—The effect of limited radiant energy (sunlight) and a rachitic ration. This bird is one from a group fed white corn 97, calcium carbonate 2, sodium chloride 1, and skimmed milk, ad libitum. Sunlight was limited to ten minutes per day. Weight, 140 grams at six weeks of age. It is perfectly evident that the specimen is an abnormal one. (Courtesy of Professor E. B. Hart, Wis. Agr. Exp. Sta.)

food-stuff to corn, using the chick as an experimental subject, yields results entirely comparable with those secured with the rat or with swine.

Emmett and Peacock (1922), in chick feeding experiments, employed rat rations modified by increasing the concentration of protein, and including roughage in the form of charcoal, oyster shell, or grits. The chicks were 3 to 4 weeks old. The diets were deficient in vitamins A and B. Chicks receiving diets deficient in vitamin B developed polyneuritis in 10 to 12 days and those receiving diets deficient in vitamin A lived 14 to 21 days. The latter group developed leg weakness, poor plumage, edema about the eyes, beading of the ribs, atrophy of the testes and

hypertrophy of the adrenals and gall bladder. They believe the requirements of the chick to be less for vitamin C than for A and B. Plimmer and Rosedale (1923) have demonstrated that the chick does not require vitamin C.

Pappenheimer and Dunn (1925) showed by radiographic and histologic study that young chicks with experimentally pro-

FIG. 28.—The effect of unlimited radiant energy (sunlight) and a rachitic ration. This bird was taken from a group that received white corn 97, calcium carbonate 2, sodium chloride 1, and skimmed milk ad libitum. This group was exposed to sunlight the entire day. Weight, 210 grams at six weeks of age. The group at this age appeared normal although vitamin A was in low amounts in the ration. (Courtesy of Professor E. B. Hart, Wis. Agr. Exp. Sta.)

duced leg weakness showed no rachitic changes. There was arrest of osteogenesis, osteoporosis and fibromyxomatous transformation of the marrow. The non-saponifiable fraction of cod liver oil did not prevent leg weakness nor modify the character of the bone lesions. The addition of whole cod liver oil prevented leg weakness and brought about normal bone structure.

Bethke, Kennard and Kik (1925) could not prevent leg weakness in chicks by a diet containing 18 per cent. of fresh green red clover, although the latter prolonged life, increased growth, and delayed the onset of leg weakness. On the same diet exposure to sunlight for 30 minutes daily checked leg weakness. Access to soil possibly decreased the mortality rate, but did not apparently benefit the nutrition of the chicks when other nutrients were supplied. They state that the beneficial effects ordinarily ascribed to green plant tissue and extraneous materials ingested by chicks on range are due largely to direct sunlight rather than to other factors.

Hughes and Titus (1926) state that comparison of the calcium and phosphorus in the blood of normal chicks and those showing less weakness indicate that this condition is the same as rickets. Goodale (1926) states that the amount of exposure to sunlight or ultra-violet rays necessary for normal growth in the chick is very small. Irradiation for 1 hour and 10 minutes twice weekly from the mercury lamp at 3 feet gave normal growth.

The work of Maynard, Goldberg and Miller (1925), Godden and Husband (1925), Bohstedt and co-workers (1926) and Maynard and Miller (1927) and others, have fully confirmed in principle the facts brought out by the experiments discussed in this chapter.

CHAPTER XXII

RELATION BETWEEN NUTRITION AND FERTILITY

Vitamin E

McCollum and Davis (1915) in a study devoted to the effect of the composition and amount of the mineral content of the diet on growth and reproduction, employed a diet consisting of whole milk powder 10, casein 15, butter fat 5, agar-agar 2, dextrin 53, and sucrose 15, to which various inorganic additions were made. They stated: "It was a surprise to us that all lots failed to repeat reproduction at normal intervals. This failure is certainly the result of some factor other than the character of the mineral content and must remain unexplained for the present."

Reynolds and Macomber (1921) stated that decreasing the content of the diet in vitamin A, in protein and in calcium markedly decreased fertility in rats. Evans and Bishop (1922) described a disturbance of the estrus in adult female rats when the diet was deficient in vitamin A. They studied several nutritive regimens adequate for livelihood, for approximately normal growth and for the appearance of health, but which were inadequate for the maintenance of the normal rhythm of ovulation. Animals reared on diets consisting largely of purified food substances and an abundance of vitamins A and the water-soluble group then called B, failed of reproduction in a large proportion of cases. Small amounts of some natural foods cured the sterility thus produced, showing that the disorder was due to dietary deficiency. They demonstrated the possibility of extracting from natural foods material which was very concentrated in the specific substance concerned with the promotion of fertility, and presented adequate evidence for establishing the existence of a substance now called vitamin E. This vitamin is fat-soluble and is especially abundant in certain fats of vegetable origin, notably the oil of wheat embryos.

Vaginal Changes During the Estrual Cycle.—Evans and Bishop showed that the precision with which one may detect the periodicity in ovarian function in the rat is remarkable. Several

investigators had previously described definite changes associated with the phenomenon of heat, or rut, in several common types of mammals. Stockard and Papanicolaou (1917) showed that microscopic examination of the cell detritus and exudate in the vaginal canal enable one to determine the exact time of estrus and ovulation and to segment the estrual cycle into definite periods or steps. Evans and Bishop described in detail observations which show that in the rat several periods in the estrual cycle recur with striking regularity every 4 or 5 days, during which the mucous membrane of the vaginal canal exhibits a period of growth, differentiation and desquamation in so orderly a sequence as to furnish accurate information about the approach and incidence of ovulation.

Irregularly shaped, small, nucleated epithelial cells mixed with leukocytes form the picture in the resting stage. Preparatory to estrus and ovulation there is a pause in the immigration of leukocytes which find their way in considerable numbers through the epithelial cells of the vaginal mucosa into the lumen. Leukocytes are suddenly no longer encountered in the smear. Epithelial cells alone, sometimes in sheets, are now found. Quickly succeeding this change come non-nucleated, transparent cornified cells, sooner or later in similar sheets. A massive production of these cells takes place so that macroscopically a cheesy detritus is found. Eventually leukocytes again recur and quickly thereafter are found in enormous numbers. The cornified epithelial cells are replaced by small nucleated ones which are not abundant. This forms the cycle of changes. The presence of true cornified cells, singly or in sheets, is a reliable criterion for impending maturation of ova and consequent rupture of Graafian follicles in the ovary.

Effect of Vitamins A and B on Ovulation and Estrus.— Employing rats fed upon a diet consisting essentially of casein, corn starch, lard, butter fat, a salt mixture, and a small amount of dried yeast to supply the water-soluble vitamins, it was noted that while all other phenomena were apparently normal, the animals showed late maturity and infrequent ovulations. With larger doses of yeast (100-600 mg.) daily the ovulation rate became normal. This new test for physiological well-being was therefore more sensitive and more significant than that furnished by growth, appearance of coat, bodily activity, longevity, etc.

When the experimental diet was impoverished in vitamin A, a new and characteristic sign of a lack of this vitamin was dis-

closed,—the tendency of the vaginal mucosa to form cornified epithelial cells was no longer limited, as is normally the case, to the time of growth, maturation and rupture of the Graafian follicles, but in the A deficient rats the desquamation of cornified cells was continuous and obscured all ovarian cycles that may also have been present. Vitamin A deficiency does not create a mal-function of the ovaries which continue to secrete hormones similar to those in estrus. For this new sign Evans and Bishop offer the probable explanation that A deficiency produces xerosis of the epithelium of the vaginal membrane, as it was previously shown to do in the lacrymal gland, causing a cessation of tear production. By an examination of the vaginal smear they found it possible to detect vitamin A deficiency in otherwise apparently healthy animals receiving enough A to prevent ophthalmia. Raising the level of A immediately abolished the sign.

Wolbach and Howe (1925) have reported on the extensive transformation of epithelium in various parts of the body into a stratified, squamous keratinizing epithelium, particularly of the upper respiratory tract and in the renal pelvis, urinary bladder, seminal vesicles, epididymis and prostate, as well as the salivary glands and pancreas, resulting from A deficiency.

The sterility disease described affects males and females differently; in the male it leads to destruction of the germ cells and eventually of the entire seminiferous epithelium, producing permanent reproductive incapacity, whereas in the female the ovary and ovulation are unimpaired throughout life. There is, however, a highly characteristic disturbance in gestation, caused by the death and resorption of the developing young.

Many other dietary deficiencies cause sterility in the female, but this sterility is the result of interference with other steps in the reproductive mechanism than those involved in lack of vitamin E, usually by preventing estrus, ovulation, fertilization, or implantation, whereas the E deficiency sterility is due to resorption after implantation has occurred.

Evans and Bishop established female sterility as due to E deficiency by demonstrating the existence of estrus, ovulation, coition, and implantation. In gestation where E is insufficient or absent the embryos at first appear normal, but show retardation in development by the eighth day or soon thereafter. There is no detectable abnormality of structure. At some time between the twelfth and twentieth day fetal death occurs, usually on the twelfth or thirteenth day. The maternal placenta is not altered

structurally sufficiently to indicate that its function is impaired. The fetal parts of the placenta, both in yolk sac and allantois, show impaired development. In the yolk sac there is underdevelopment of the entodermal villi and blood islands, whereas in the embryo there is impairment in the mesenchyme, the blood-vessels and blood-cells. Dead and living young may occupy neighboring sites in the same uterine horn.

Distribution of Vitamin E.—Vitamin E is a fat-soluble principle which occurs in muscles, fat, pancreas, spleen, liver, heart, hypophysis, placenta, and other structures. It is said to be less abundant in the viscera, including the liver, than in muscle. Heart, spleen, brain, kidney and testes contain little E, whereas muscles and fat contain it in considerable amounts. Milk fat is relatively a poor source of it, since 9 per cent., together with 15 per cent. of lard, failed to prevent sterility, though with lard absent 24 per cent. of butter fat succeeded. Milk from cows on fresh alfalfa pasture is richer in E than milk from dried feeds. According to Evans and Bishop, cod liver oil, which is rich in A and D, is notably lacking in E. In their experiments 9 per cent. of cod liver oil in the ration throughout the life of the rat permitted sterility to develop, although a single drop daily was adequate for A requirements.

The seeds and green leaves from plants are good sources of vitamin E. Even dried lettuce, alfalfa, pea, etc., provide it. Twenty-five grams daily of lettuce leaf powder cured sterility in rats. The vitamin is present in corn, oats and especially wheat; it is low in the endosperm but abundant in the oil of the embryo or germ. Ether extraction of lettuce leaf or wheat germ removes E quantitatively and furnishes preparations of which 25 mg. daily is sufficient to insure fertility. They also report that cottonseed oil when hydrogenated is practically devoid of A but contains enough E to support fertility in the rat. Corn oil, olive oil, coconut oil, walnut, peanut and flaxseed oils were fed in quantities 5 times the required minimum of wheat germ oil without restoring fertility.

Experiments in which animals were kept on an E deficient diet and given as a supplemental food the tissues of other rats reared on an E deficient diet consistently showed sterility, indicating a lack of E in the tissues fed. Supplementing the sterility diet with tissues of rats reared on natural food induced fertility.

Survival of Fertility in Animals Reared Normally and Changed to a Diet Deprived of E.—Animals reared on a diet of

natural food-stuffs, and after fertility was established shifted to a pure food ration, preserved their fertility for 3 or 4 months, after which time they became sterile. When sterile animals are cured with foods containing E the next gestation is normal, and even the next 2 or 3. The survival of normal fertility is dependent on the amount of E in the curative diet.

Vitamin E is transferred from mother to offspring during intrauterine life, for the tissues of newborn rats cures female dietary sterility. The body stores of vitamin E are used up in normal metabolic processes at approximately the same rate, whether or not there is the strain of gestation.

A remarkable observation of Evans and Bishop was that curative foods or extracts of them could be fed as late as the fifth or sixth day of pregnancy and save the situation, so that normal gestation resulted. Subcutaneous or intraperitoneal injection of the vitamin is as effective as feeding by mouth.

Evans (1924-1925) states that vitamin E is soluble in methyl and ethyl alcohols, ether, pentane, benzene, acetone, ethyl acetate, and carbon disulfide, as well as in fats. It is almost insoluble in water. It is remarkably stable to heat, light, air, and many of the ordinary chemical reactions. Distillation of wheat germ oil, or a preparation made from it, in super-heated steam at 180° C. for several hours did not destroy it. Distillation *in vacuo* up to 230° C. did not lower the potency of the fraction so treated. Wheat germ oil exposed to air at 97° C. did not lose its E content in 12 hours. It is remarkably stable to both acids and alkalies, and is unchanged by exposure to saturated alcoholic hydrogen chloride. Hydrogenization was without effect. Alcoholic extracts of hydrogenized cottonseed oil were fairly rich in E. Twenty per cent. hydrochloric acid in contact with wheat germ oil for 20 hours at room temperature was without destructive effect. It resists the action of boiling 20 per cent. alcoholic potassium hydrate, though partial destruction occurs on very prolonged hot saponification. Saponification with 20 per cent. alcoholic potassium hydrate can be carried out at 30° C. without great loss of the vitamin, which goes into the non-saponifiable fraction of the oil.

Considerable difference of opinion has arisen among investigators as to the existence of a fertility vitamin. The views are based in great measure on the fertility of rats on diets in which milk powder formed the principal component. Palmer and Kennedy (1923) observed sterility in rats confined to raw liquid

milk supplemented with water and iodine. The rats exhibited no mating instinct. Females were reared to partial maturity on a mixed diet, after their first litters they were placed on the raw milk diet, but subsequently produced only unhealthy young.

Studies on Skim Milk Powder.—Sure (1924) used a diet containing 50 per cent. of skim milk powder plus an iron salt. Normal reproduction was not secured until oil of yellow maize equivalent to 5 per cent. of the diet was added; this he interpreted to mean that the oil provided vitamin E. Cottonseed oil and olive oil were also effective, but coconut oil, linseed and sesame oils were not. Benzene, acetone, and ether, extract E from grains. He found that olive oil, peach kernel oil, soy bean and peanut oils induced fertility but failed to support lactation. Cottonseed oil induced fertility and was partially successful in lactation, whereas wheat germ oil, hemp seed oil and yellow corn oil were potent for both purposes. Other oils found deficient in E were rape seed, mustard seed, and oil of sweet almonds. In wheat oil he believes there are two vitamins, a heat-stable E and a heat-labile one which is associated with lactation. This view recalls the work of Benedict (1918) and Sugiura and Benedict (1918) who concluded that the milk produced by a rat confined to a diet of banana, casein, salts, yeast and carrot extract was deficient not only in quantity but also in quality. They were the first to suggest the existence of a vitamin concerned with lactation. Grijns and de Haan (1926) also report experiments which support the view that there is a lactation vitamin. Much further work must be done to clarify the situation.

In further support of the existence of vitamin E, Sure (1927) reports evidence that as little as 0.0175 per cent. of the unsaponifiable matter from crude cottonseed oil added to a skim milk diet prevents female sterility, and double this amount had a favorable influence on lactation. Sure, and Evans have both stated that cod liver oils containing much A and D are deficient in E. Simmonds, Becker and McCollum (1928) fed rats diets of purified food substances, supplemented with yeast, and lard, and cod liver oil, and secured fairly high fertility and normal deliveries. Evans expressed the belief that lard has an anti-E effect, but the results just reported seem convincing that there is some favorable effect of feeding cod liver oil and lard together. Females invariably died at delivery when cod liver oil was the only fat provided, and were normal when lard was combined with it. Even a combination of wheat germ oil 2, and cod liver oil 2

per cent., resulted in death of females at parturition when the diet contained no other fat. Lard as the sole fat in the food could not be tested since it furnishes no A and the health of the animals could not be maintained. Either lard 15, with 9 per cent. of butter fat, or cod liver oil 8 combined with butter fat 10 per cent., gave normal nutrition and normal deliveries. It is obvious that the beneficial effects of feeding together lard and cod liver oil are due to each oil providing something indispensable for normal delivery. The supplementary effect may be due to a small content of E in each, or possibly to each containing a different vitamin in some manner associated with fertility.

The experiments just discussed are to be considered together with those of Anderegg, Nelson, and others (1924, 1926) who observed reproduction to the fourth generation in rats fed whole milk powder as the sole source of vitamins. In most of their studies reproduction was not normal, and they interpreted this to be due not to deficiency of E, but to unsatisfactory content of milk in respect to several known constituents. The discovery by Hart and Steenbock (1928) of the deficiency of milk in copper and its importance in the assimilation of iron, may have determined the outcome of certain of their studies, since some milk samples may have been contaminated with both copper and iron by pasteurization or powdering machinery. The importance of some specific substance (E) present in certain fats for reproduction appears to rest on a sound experimental basis.

Mattill, Carman and Clayton (1924-1925), Evans (1925), Parkes and Drummond (1925) and Mason (1926) have studied in male rats sterility induced by diets deficient or lacking in vitamin E. Evans found that young males from mothers which had had an abundance of E, if weaned on the twenty-first day and fed a diet lacking in E, were sterile after the fourth month. The sterility is described as occurring in four stages: (1) normal abundance of sperm with loss of fertilizing power, but with approximately normal sex responses; (2) complete absence of sperm with sterility; (3) loss of power to form the vaginal plug, and (4) loss of all sex interest. Mason states that the degenerative process takes place in 35-50 days. After degeneration of the germinal epithelium has set in, administration of lettuce (E), could not completely restore the testes to a normal condition. Mattill also found sterility induced by deficiency of E to be permanent even when an abundance of the vitamin was fed after testicular degeneration had occurred.

Parkes and Drummond (1925) have discussed the sterility resulting in rats from deficiency of vitamin B. There was degeneration of the testes, which were not restored by a normal diet. The fecundating power of males was greatly depleted before sterility occurred. Parkes and Drummond (1926) also discussed the sterility induced in male rats by vitamin A deficiency. The testes, though stunted, were fully developed. The sterility was attributed to physical debility and disinclination to copulate. There was full recovery when health was restored by provision of A. Mattill (1927) discussed the sterility resulting from B deficiency, and expresses the belief that in reality it is due to E deficiency only. Rats depleted by B starvation to a point of helplessness from paralysis showed normally motile sperm in the epididymis. He regards the sterility due to B deprivation to be due to lack of sex expression. Evans (1928) has also reported on the effect of inadequate vitamin B upon the sexual physiology of the male. He concludes from his studies that both acute and chronic deficiency in the anti-neuritic vitamin—deficiency leading to death—leaves the anatomical and functional integrity of the male germ cell unaffected. Sex interest is decreased but this can only be shown a few days before death. If vitamin E is provided in sufficient amounts, injury to the productive function in the male caused by inadequate B appears, he says, to be due solely to this interference with behavior and to no extent to actual injury of the male germinal elements. Evans (1928) has also studied the relation of vitamin E to growth and vigor. His results show that vitamin E does not exert its favorable effects upon growth and the general state of the animal indirectly through its established value to the sex glands, but in some other more direct way. This question is being studied further. The recent work of Mason (1929) suggests that possibly vitamin E consists of two parts; but more work is necessary before this can be established.

For a complete discussion of the relation of nutrition to sterility up to 1927 the reader is referred to the valuable monograph of Evans, Burr and Althausen (1927).

CHAPTER XXIII

THE RELATION OF PELLAGRA TO DIET

Vitamin G

Early History.—Pellagra was described in northern Spain by Casal in 1735, but for many years it was most common in parts of Italy. Aside from Italy and Spain, pellagra has been prevalent during the last century in parts of France, the Balkans, especially Roumania, and for a lesser time in Egypt. It has afflicted, still more recently, many people in the United States, where it was not recognized until 1908, but from that year it rapidly increased, until by 1917 there were recorded 170,000 cases of pellagra, principally in the Southern States.

Symptoms.—Pellagra is a disease involving the nervous system, the digestive tract and the skin. Usually one of the first symptoms is soreness and inflammation of the mouth, followed by the appearance of remarkably symmetrical erythema on various parts of the body. The nervous symptoms become gradually worse as the disease progresses. The spinal cord is especially the seat of injury, but the central nervous system is also involved in many instances.

Theories Concerning Etiology.—Several views have been proposed to explain its etiology. Marzari, more than a century ago, expressed the belief that pellagra resulted from the excessive use of maize as food. Italian investigators later accepted the view that it was caused by eating moldy maize. Two other theories have been advanced to account for its etiology: one, that it is caused by an infecting organism, and the other, that it is in some way related to faulty diet. None of these views has been established by entirely satisfactory evidence, but the data now point incontrovertibly to a relation between pellagra and the diet. One group of investigators believes that pellagra is an infectious disease, but admits that lowered vitality from faulty nutrition is an important predisposing factor. Another group holds that the syndrome results from the lack in the diet of a specific substance or substances, and that therefore pellagra is a deficiency

disease. Since almost all students of this disease are agreed that there is some relation between the character of the diet and susceptibility to it, the present chapter will be limited to a discussion of the evidence of the rôle of nutrition as an etiological factor in pellagra. Roussel (1845) stated that the most effective treatment of pellagra is a milk diet. Lussana and Frua (1856) studied the effect of improvement of the diet of pellagrins, and reported that they had reduced the mortality in about eight thousand cases from 24.5 to 4.5 per cent., and had increased the rate of recovery from 20 to 70 per cent. The idea that the disease could be prevented or cured by proper diet dates back many years.

Wussow and Grindley (1911) emphasized that the diet of the insane patients under their observation was chiefly composed of vegetables and that it had a low content of animal protein. At that time the significance of the proper selection of food for the promotion of well-being was not understood.

The Thompson-McFadden Commission, consisting of Siler of the United States Army, Garrison of the United States Navy, and MacNeal of the New York Post Graduate Medical School (1913), made an epidemiological study of pellagra in Spartanburg County, S. C. They collected data regarding the diets of pellagrins. The information was obtained from statements of physicians, patients, storekeepers, millers, neighbors, and others. Their investigations (1914), which were extended to include an examination of the dietaries of non-pellagrins, were interpreted to mean that there is no relation between the character of the diet and the incidence of pellagra. The Commission concluded after several years of observation that "pellagra spread from a preëxisting case as a center in the six villages studied," and that "pellagra morbidity was higher in congested communities using surface privies than in more sparsely settled districts in which similar methods for the disposal of excreta were employed." Further, " . . . the endemic foci of pellagra were located in the districts in which surface privies were in use." In two cotton mill villages completely equipped with a water-carriage system of sewage disposal it was impossible to find cases of pellagra which had originated there, although some cases which had originated elsewhere were present. It was decided that the stable fly (Stomoxys calcitrans) displayed certain salient characteristics which seemed to qualify it as a vector of pellagra.

Relation of Diet to Pellagra.—Goldberger (1914) noted in institutions where pellagra was either epidemic or endemic

among the patients, that the physicians, nurses and attendants were almost without exception immune; this indicated that contact with pellagrins or opportunity for transmission by bedbugs did not seem a satisfactory explanation for the transmission of the disease. In institutions where the food purchases were assumed to be alike for patients and attendants, it was found that the diets of the two groups were not comparable. The staff chose the best articles, and diverted certain foods to their own table when the amounts did not suffice for all. The patients were at a disadvantage in respect to food. Where the meat and milk supplied for an insane asylum were inadequate, the milk and the desirable cuts of meat found their way to the table of the staff, while gristle and the unattractive pieces of fat were served to the patients. Physicians, nurses and attendants were also in a position to supplement the institutional diet.

Goldberger found in certain institutions where pellagra was common that more cereals and vegetables were used than in the dietaries of people in better financial circumstances who were practically immune to the disease. This led him to undertake to prevent the disease in institutions by improving the dietary.

At this time little was known of the properties of the different natural food-stuffs, which subsequently have become known through the systematic studies of individual foods by means of the biological method of analysis introduced by McCollum and Davis (1915). Goldberger compared the diets of persons who developed pellagra with those living in the same region but remaining free from the disease. Those who remained free from the disease ate more fresh meats, eggs and milk than those among whom the incidence was high. The well-to-do took a better diet throughout the year than did the poorer people, whose food supply suffered greater seasonal changes. The winter diet of the latter was simpler and more monotonous. The recognition of the importance of seasonal variations in the diet as an etiological factor in pellagra was one of great importance. It is true that pellagra is in great measure confined to the poorer classes, and to those who live in the less desirable parts of cities and villages. It occurs occasionally among the well-to-do, but much less commonly than among those who depend upon a daily wage.

Goldberger, Waring and Willett (1915) carried out an experiment in two orphanages and in an insane asylum to test whether pellagra could be prevented or cured by proper diet. They added liberal amounts of fresh meat, eggs, milk, and fresh vegetables to

the institutional diets since these diets differed from the better class American diets in the absence of these foods. In one of the orphanages under observation during 1914, there was no recurrence of pellagra in any of the 67 persons afflicted, who were under surveillance at least until the anniversary of their attack. Among 99 pellagrin residents who were under observation for at least a year there was not a single case of the disease after the change in the diet. At the other orphanage 105 pellagrins were observed for at least a year, including the anniversary of their attack, and there was not a single case of recurrence. Sixty-nine individuals who had not suffered from the disease were observed for at least a year and among these not a single case developed.

At the Georgia State Asylum, which was an endemic focus of pellagra, the diet of two wards was greatly improved, but no change was made in hygienic or sanitary conditions. In these two wards 72 patients suffering from pellagra during 1914 were kept under observation at least beyond the anniversary of their last attack, but not one of them showed any signs of recurrence. During the corresponding period, 47 per cent. of 32 control patients in another ward showed definite recurrences. The conclusion was drawn that pellagra can be prevented by an appropriate diet, even when the hygienic conditions are unfavorable.

The Prison Farm Experiment.—Goldberger (1915) planned an experiment to determine whether a faulty diet of the type common among pellagrins would produce the disease in man. The plan involved restricting men to the type of diet characteristic of the homes of the cotton mill workers throughout the section of the South where pellagra was very common. The experiment is of extraordinary interest because of the care with which it was executed, and because of the skill of the observers.

Pardons were offered to any healthy white men in the state prison who would offer themselves as experimental subjects; twelve men volunteered and 11 underwent the test. White male adults were selected because this group of the population in pellagrous districts had a lower incidence of the disease than any other race, sex or age. It was believed that the experiment, if successful in causing pellagra, would be the more conclusive because carried out with this selected group of subjects.

The experiment was started at a season of the year when, if pellagra was successfully produced after a period corresponding approximately to the winter season, it would appear out of season, i.e., in the autumn. The men were placed on the experi-

mental diet on April 19, 1915, and continued on it until October 31 of the same year. The experiment was carried out on the prison farm. Of 180 convicts on the farm at the beginning of the experiment, 30 were present until it was terminated. No cases of pellagra occurred on the farm during the experiment, and none had been noted among the convicts or attendants at the institution in previous years, although it was fairly prevalent in the surrounding country. The 11 experimental subjects were carefully protected against exposure to pellagra, since they were under guard throughout the experiment.

The diet of the experimental group consisted of white wheat flour, degerminated corn meal (maize), polished rice, starch, sugar, molasses, pork fat, sweet potatoes, collards, turnip greens, and coffee. From data furnished by Doctor Goldberger, we calculated that less than 4 per cent. of the total energy value of the diet was derived from cabbage, collards, turnip greens, and sweet potatoes. The diet was therefore essentially derived from degerminated cereal products, together with molasses and fat pork. It will be recalled it has been demonstrated that such a diet is inadequate for the maintenance of health in adult animals. The average protein intake was 41-54 gm.; of fat, 91-134 gm.; and of carbohydrates, between 387 and 513 gm. per man per day. At the end of five and a half months 6 of the 11 men were diagnosed as showing the skin lesions characteristic of incipient pellagra; the diagnosis was concurred in by a number of dermatologists familiar with pellagra.

Goldberger and 15 of his associates (1916) next tried in a number of ways to transmit pellagra to themselves by inoculations with blood, nasopharyngeal secretions, feces, urine, and desquamating epithelium. The results of this heroic experiment were entirely negative: not a single one showed any evidence of the disease. These subjects were all presumably nourished on a satisfactory diet previous to and during the experiment. The results of this experiment could be interpreted as evidence that pellagra is a disease of low virulence and that it is liable to attack only that portion of the population with lowered resistance to infection.

Similarities Between Scurvy, Beri-beri and Pellagra.—Vedder (1916) states that "there is a certain similarity between pellagra and other known deficiency diseases, namely, beri-beri and scurvy," and "much of the evidence that has been presented as a proof of the infectious nature of pellagra can be reasonably

FIG. 29.—Illustrates the skin lesions on the arm and hand of a pellagrin.—Courtesy of the Thompson Pellagra Commission.

explained in accordance with a deficiency hypothesis." He further concludes: "A deficiency is demonstrable in the diets of most pellagrins. This deficiency appears to me to result from the too exclusive use of wheat flour, in association with corn meal, salt meats and canned goods, goods that are known to be deficient in vitamins." He also states: "The gastro-intestinal lesions in pellagra and scurvy are analogous. Diarrhea, enteritis, ulceration of the intestines and hemorrhage into the mucous membranes are observed in both conditions. There are similar nervous symptoms in pellagra and scurvy."

He further points out that suggestive similarities exist between pellagra and beri-beri. The mucous membranes of the stomach and duodenum may be swollen and inflamed, and may present ecchymoses and erosions. "Similarities in the lesions in the nervous system and in the symptomatology referable to the nervous system in pellagra and beri-beri can be distinguished. The pathologic alterations that occur in the cord in pellagra are profound and striking." Vedder also states: "Now, if we compare this picture with the changes found in the cord in beri-beri, we find that beri-beri is characterized by the same scattered degeneration of the fibers and similar changes in the cells of the cord." In beri-beri there is degeneration of many of the motor cells in the cord. Roberts (1912) also describes the extensive degenerations which have been observed in the cord in pellagra.

Vedder points out that central neuritis symptoms are very common in pellagrins, and that exactly such changes in the nervous system caused beri-beri for many years to be regarded as an intoxication. To quote further: "The fact that the spinal fluid in pellagra is normal, points toward deficiency, since it seems improbable that such extensive changes in the spinal cord could occur as the result of an infection without producing corresponding changes in the spinal fluid. Even the skin lesions which are so characteristic of pellagra may be referable to changes in the cord. Otherwise, how can we explain the marvelous symmetry that is practically the constant characteristic of this symptom?" These are pertinent questions to raise in connection with any decision concerning the nature of the factors involved in the etiology of pellagra.

Pellagra in Egypt.—The Director of Medical Services of the Egyptian Expeditionary Force directed that a committee of medical men in the British Army make a study of the prevalence of pellagra among Turkish prisoners of war. This report was made

by Boyd and Lelean (1919), who attributed pellagra to starvation for protein, and especially to a low intake of protein of low biological value. The occurrence of pellagra among those who had never eaten maize could be explained as a consequence of their securing, through some dietetic error, a protein supply of low biological value.

Bigland (1920) says in relation to the pellagra outbreak in Egypt, "From the experimental side all efforts to obtain organisms from the blood and feces on various media, complement fixation with alcoholic extracts of organs obtained at autopsy, inoculations of rabbits with pellagrous sera and the diagnostic use of maize extract, failed of positive results." The diets of the pellagrins showed a deficiency in the "biological value of proteins," but there were a few cases in which no such deficiency could be proved.

Studies of Voegtlin.—Voegtlin (1920) tested on over one hundred pellagrous patients the effects of two types of diets on the clinical condition of the disease. Patients with a moderate attack of pellagra uncomplicated by any other disease were placed upon a diet which closely resembled that to which they were accustomed before the attack. This will be designated as diet X. It consisted of the following ingredients:

White bread	300	grams
Butter	30	"
Cabbage	100	"
Corn meal (maize)	50	"
Ham	25	"
Hominy	75	"
Corn syrup	75	"
Pork	50	"
Potatoes	150	"
Prunes	30	"
Turnip tops	100	"
Sugar	40	"
Milk	40	"

This diet contained 50.5 gm. of protein; 89.8 gm. of fat; 350.8 gm. of carbohydrate; and yielded 2473 calories of energy.

Voegtlin says: "Almost without exception the general clinical condition of these patients remained either stationary or gradually became more aggravated simultaneously with an increase in the pellagrous manifestations. The skin lesions often spread to parts of the body which had not been affected previously; there was also an increase in the stomatitis and the gastro-intestinal symptoms. The appetite, as a rule, was good for the first few weeks, but diminished gradually. The nervous manifestations, such as disturbances in sensation, reflexes, and mentality, either

showed no change or increased in severity. A few cases developed an acute psychosis. A careful examination of the dietary record showed that the patients had consumed sufficient food." The patients were changed after a time to diet Y, the composition of which was given as follows:

White bread	300	grams
Butter	45	"
Corn meal	50	"
Eggs	100	"
Meat	100	"
Orange juice	100	"
Potatoes	150	"
Prunes	30	"
Sugar	40	"
Milk	1000	"

This diet contained 102.9 gm. of protein; 115.3 gm. of fat; 316.2 gm. of carbohydrates; and furnished 2781 calories of energy.

On diet Y, which differed from diet X in containing 4 eggs, 100 gm. of fresh beef and one liter of milk, the patients showed gradual improvement, ending in many cases in the complete disappearance of all recognizable manifestations of the disease. Another group of patients which was placed on diet Y immediately upon their admission to the hospital, showed definite improvement within 2 weeks. Within 2 months the patients were judged to have recovered from the disease, except in a relatively few advanced cases which showed no benefit from the improved dietary.

Voegtlin next placed patients on diet X for a time until it was observed that their clinical condition remained stationary or grew gradually worse. Some were then given one of the following extracts of natural food-stuffs:

(1) A fat-free, alcoholic extract of yeast or rice polishings.

(2) A fat-free, alcoholic extract of ox liver or thymus gland.

The first of these extracts was chosen because it contained an abundance of vitamin B; the second contained a relative abundance of vitamins A and B. The results indicated that the administration of extracts of yeast or rice polishings did not modify the course of the disease in patients maintained on diet X. Voegtlin states that "the administration of the liver preparation to pellagrins was followed by an improvement in their condition, apparently comparable to that produced by the consumption of a diet containing a considerable amount of milk, eggs and meat. The evidence so far available, therefore, indicates that the dietary defect presumably responsible for pellagra is distinctly different from, and probably more complex,

than the one causing human beri-beri." From recent work on pellagra, it would appear that the fat-free, alcoholic extract which Voegtlin used contained the factor G, the absence of which causes pellagra.

Pellagra in Infants.—Voegtlin and Harries (1920) reported pellagra in infants who were nursed by pellagrous mothers, and cite older literature which shows that a considerable number of cases of pellagra have been observed in infants. A mother suffering from severe pellagrous lesions was admitted to the hospital while nursing a seven-months-old infant. The child did not show symptoms of the disease. The mother was placed upon Diet A for 35 days. This diet is of a type commonly used by pellagrins.

DIET A

Breakfast: Hominy, 75 gm.; butter, 15 gm.; corn syrup, 30 gm.; wheat bread, 100 gm.; coffee, with 20 gm. of sugar; milk, 20 c.c.

Dinner: Potatoes, 150 gm.; cabbage, 50 gm.; turnip tops, 50 gm.; fat pork, 30 gm.; wheat bread, 100 gm.

Supper: Corn meal mush, fried in pork fat, 50 gm.; lean boiled ham, 25 gm.; prunes, 30 gm.; wheat bread, 100 gm.; coffee, with 30 gm. of sugar; milk, 20 c.c.

Samples of milk were secured from this patient, and from four others on the same diet, and these samples were analyzed by the most approved chemical methods. The lactose, fat, total nitrogen, and total solids were found to fall within normal limits, but were below the average; the ash and phosphorus contents were normal, whereas the calcium, magnesium and potassium were below normal. No tests were made of the vitamin content of the milks. The total volume was greatly diminished in most cases.

None of the infants of the 5 pellagrous mothers showed symptoms of the disease. It would seem that if any relation existed between pellagra and the diet of the mother that her nursing infant should develop the disease. Pellagra is uncommon in infants who are nursed by mothers suffering from the disease. Pellagrous mothers usually feed their infants the same food which they eat, when the breast milk is too scanty to suffice. This replacement of a considerable amount of mother's milk by food which leads to pellagra in the adult should, theoretically, predispose the infant to the disease, which does not seem to be

the case. No one has been able to demonstrate the presence of anything toxic in the milk or blood of pellagrins.

It is probable that the milk of pellagrous women is deficient in vitamins A, B, C, D and G, which are known to produce specific syndromes. The occasional occurrence of pellagra in infants whose mothers are suffering from the disease may be accounted for on the assumption that it is caused by lack of a specific vitamin.

Voegtlin and Harries report pellagra in an infant about one year of age. Her mother, who was undernourished and suffered from chronic indigestion, nursed her throughout this time, and showed no evidence of the disease. No other member of the family showed symptoms of pellagra. This case would seem to support the view that pellagra is due to an infection. It is of course possible that an error was made in diagnosis. It is difficult to understand from the studies made on the etiology of pellagra how a nursing infant could suffer from the disease and the mother remain free from it.

Lustbert and Birchett (1922) report that 3 breast-fed children of pellagrous mothers exhibited certain symptoms of pellagra. The mothers' milk was normal in fat, carbohydrate and protein. The diets of the mothers consisted chiefly of dried beans, dried peas, corn meal, lard, and evaporated milk; they were poor in protein, rich in carbohydrate and fat, and were deficient in vitamins and in certain inorganic elements. Usually the disease was present in the child only in a mild form and responded to dietetic treatment which consisted of removal from the breast, and feeding with cow's milk, fruit juices, vegetable purees, and albumin milk.

Other Views Concerning Etiology of Pellagra.—Viswalingam (1920) says that the deficiency and parasitic theory holds good in the etiology of pellagra among the Chinese in the Malay States. That faulty diet is not solely responsible for the disease is shown, he believes, by the seasonal recurrence of symptoms in patients placed in a hospital with adequate diets for a considerable time.

Goldberger (1920) shows that the claims of Enright (1920) and of Viswalingam (1920) that the food deficiency theory of the causation of pellagra has been exploded are based on insufficient evidence, since it cannot be assumed in the case of war prisoners that the diet furnished, although adequate, was consumed, because of the racial differences in methods of prepara-

tion, seasoning, etc. Biglands's toxin hypothesis appears to be superfluous on the same ground. Goldberger also points out (1920) that data relating to diet must be secured from individual pellagrins or non-pellagrins, and not from the family menus alone. It must be kept in mind that the seasonal changes in the diet may be of the greatest importance. He also pointed out that the condition which it is necessary for the subject to reach before a diagnosis of pellagra could be made, should be carefully stated. Conflicting data and resulting conflict of views can be explained through failure of investigators to appreciate these facts.

Wilson (1921) believed that pellagra is the ultimate result of a deficient supply of protein. Sufficiency must be judged by the amount available for assimilation, not by the gross protein. He urged that forty grams per day be taken as the minimum safe value. Sullivan, Stanton and Dawson (1921) report that either albumin or casts or both were found in about 50 per cent. of pellagrins, but some severe cases showed no kidney change. Experiments indicated poor protein absorption with high intestinal putrefaction. Goldberger believed in 1922 that the diet controlled the course and development of pellagra and that it probably depended primarily on the specific quality of the amino-acid make-up of the protein supply. He did not determine just what amino-acid, or within certain limits, combinations of amino-acids, are concerned. He stated at this time that the possibility of an unknown factor, conceivably a vitamin, alone or in combination with amino-acid deficiency, was not excluded.

Wheeler (1923) emphasizes that in addition to milk, fresh meats often stand out as the controlling factor in the development of pellagra in the individual or household. A prolonged interruption in the quantity of fresh meats consumed in the absence of an adequate supply of milk or other protein foods, frequently precedes the appearance of pellagra. The sensitiveness of borderline dietaries to what would appear to be a trifling change in some one of the meagerly provided essentials cannot be overemphasized in the prevention and control of the disease. He calls attention to several cases where a decrease or cutting off of the milk supply of a household results in the appearance of pellagra.

Intestinal Flora in Pellagra.—Jobling and Arnold (1923) observed an increase in hyphomycetes and supposed that these molds were in themselves toxic or produced a toxin when grown

on corn. They were never able to identify these molds as a causative factor in pellagra. They express the view that pellagra is not a deficiency disease comparable to beri-beri and scurvy, but one in which a definite intoxication, arising probably from the intestinal tract, forms the basis of a pathological condition. They isolated fungi which produced fluorescent substances from the feces in 5 of 9 acute cases of pellagra, and in 2 of 23 so-called chronic cases. Examination of the feces of 50 non-pellagrous persons from Nashville and Memphis did not reveal the fungus in a single case. A fluid medium in which the fungus was grown for 2 or 3 weeks became light brown and strongly acid in reaction; when made slightly alkaline it became strongly fluorescent. The fluorescent substance is soluble in lipoid solvents, and can be isolated from the medium by means of ether or ethyl acetate, and removed from the solvent by weak alkalies.

Photodynamic experiments were conducted with the extracts, which were found to be active on a potassium iodid-starch mixture in the light, but inactive in the dark. When mice were inoculated, those exposed to the light soon developed edema and reddening of the ears, and edema and swelling of the eyelids. In a few experiments death quickly followed exposure to the sunlight. If inoculations were conducted daily with frequent exposure to light the ears became gangrenous and dropped off. The tail became rough and scaly and there was some loss of hair from the face and head. These lesions are suggestive of the skin changes on the exposed parts of the body in pellagra and the results suggest that pellagra may be due to a sensitization to light through an absorption from the intestinal tract of fluorescent substances produced by an abnormal intestinal flora which persists in the intestinal tract when the diet contains excessive amounts of carbohydrates. Jobling and Arnold do not express a definite belief that this is the etiological factor in pellagra. This work was reported in 1923 and no further work appears to have been done upon it.

Hindhede (1923) asserts that pellagra cannot be due to deficiency in amino-acid supply in the diet. This view he bases upon experimental observations on soldiers and other men who lived for many months on diets which were very poor in protein. His assistant, Madsen, lived (a) for one year on potatoes and margarine; (b) for 6 months on whole rye bread, margarine, prunes, starch, and sugar; (c) for 6 months on barley meal

porridge made from water, margarine, and sugar; and (d) for 2 years on bread, potatoes, cabbage soup, and fruit soup without fats. No other investigator has reported observations on human subjects who have remained in a state of health for long periods on such remarkably simple and monotonous diets, so poorly constituted with respect to several dietary factors. It is difficult to understand, for example, why Madsen did not suffer from scurvy if he actually lived for so long a time on cooked foods only.

Pathology of Pellagra.—Denton (1925) has reported on the pathology of human pellagra. His study included 16 cases studied clinically and at post-mortem at Panama; four cases were rejected because they did not have all the clinical symptoms. The 12 selected for study had the characteristic skin lesions, lesions of the tongue, gastro-intestinal disturbances and symptoms referable to the nervous system. He concluded that the cutaneous lesions gave no evidence of being of an infectious nature. The changes in the alimentary tract do not appear to be simple, inflammatory changes. He believes that some underlying and contributory factor must be necessary for their production. He states that pellagra is a disease in which the lesions are confined to epithelial surfaces without extension into deeper tissues or organs. The cutaneous lesions are difficult to classify dermatologically. In the evolution of the skin lesions Denton says the following stages can be recognized: the stage of injury, or fibrolytic stage; reaction or the dermatitic stage; repair; compensatory vascular ectasis, or the erythematous stage; and the atrophic stage.

The sequence and character of the cutaneous changes are consistent with the theory that they are photodynamic effects. They do bear a slight resemblance to the lesions of X-ray dermatitis. The photodynamic hypothesis could be very satisfactorily applied to the cutaneous lesions if it were not for the fact that the reaction in the tongue, pharynx and esophagus is essentially the same. Denton concludes that some other factor than radiant energy must be responsible for the reaction in the skin and mucous membranes.

Attempts to Produce Pellagra in Animals.—Chittenden and Underhill (1917) restricted dogs to a diet consisting of crackers (bolted wheat flour), peas, and cottonseed oil, and produced in them a condition strikingly suggestive of pellagra in man. They developed inflammation of the mouth with sloughing of the

mucosa, diarrhea, and skin changes of a nature regarded as analogous to those seen in pellagra.

McCollum, Simmonds and Parsons (1917 and 1918) fed young rats the diet of Chittenden and Underhill supplemented in various ways with protein, mineral salts, and vitamin A. They also fed the prison diet of Goldberger supplemented by purified proteins, salts, and vitamins A and B. The results showed that these three types of corrections were all that was necessary to make the diet complete for growth in the rat. At the time these experiments were conducted it was not known that B consisted of two parts, so the P-P factor (vitamin G) was added unknowingly.

Chick and Hume (1920) fed monkeys on a low protein diet, the proteins of which were of low biological value. The animals developed skin lesions which resembled pellagra, were bilaterally symmetrical and, as in pellagra, appeared to be heightened by direct sunlight. Nervous symptoms were not observed.

The Discovery of Vitamin G (P-P).—In the course of an investigation on the influence of dietary deficiencies on experimental tuberculosis in the rat, Smith and Hendrick (1926) employed a diet of rolled oats 40, casein 10, supplemented with vitamin A in butter fat, and inorganic salts. A similar diet had been employed in 1917 by McCollum, Simmonds and Pitz, to illustrate that rolled oats required supplementing only with a protein (casein), sodium chloride, a calcium salt, and vitamin A, to render it biologically complete. The diet of Smith and Hendrick does not promote normal growth, but does so when supplemented with yeast. Smith and Hendrick sought to find whether it was yeast protein, water-soluble vitamin or some unknown factor supplied by yeast which improved the ration. They made the important discovery that yeast, autoclaved at 15 lbs. pressure until all vitamin B (anti-neuritic) was destroyed, still supplemented the oat kernel effectively, whereas the diet supplemented with proteins such as casein, gelatin, or yeast protein, but deriving all its water-soluble vitamins from 40 per cent. of oats, could not support optimum growth. Supplementing the diet with vitamin B picrate (Seidell's preparation) did not improve it. The deduction was warranted that yeast contains some substance other than protein, inorganic salts or vitamin B, which is essential for the nutrition of the rat. This substance is present in rolled oats in inadequate amount.

Salmon, Hays and Guerrant (1928) have reported on the

dermatitis of experimental pellagra in rats. They review the literature on the attempts of various investigators to produce pellagra in these animals. They describe the lesions produced and the theories set forth to explain them. They describe a dermatitis which is cured by giving a preparation known to contain vitamin G, the P-P factor. Their photographs are of interest since the condition which they show has long been observed, even in stock animals. They state that a Gram-positive coccus was constantly associated with the disease. The organism was isolated in pure culture from the skin lesions, the arthritic lesions, the parenchymatous organs, and the walls of the intestine of pellagrous rats. The organism has been fed to rats and the characteristic lesions produced; the organism has been recovered from these lesions. They believe that rat pellagra cannot be explained on the basis of a simple deficiency.

Chick and Roscoe (1928) describe in detail a procedure for the biological assay of the anti-pellagra vitamin G (B_2). Their studies confirm the conclusions of Goldberger concerning the dual nature of what was formerly called vitamin B.

Goldberger and Wheeler's Studies on "Black Tongue" in Dogs.—Goldberger and Wheeler (1928) discussed experimental "black tongue" in dogs and its relation to pellagra in man. "Black tongue" is produced by feeding the following diet:

Whole maize meal (white)	400 gm.
Cow peas (California black eyed)	50 "
Casein (leached with acidified water)	60 "
Sucrose	32 "
Cottonseed oil	30 "
Cod liver oil	15 "
NaCl	10 "
$CaCO_3$	3 "

There is an initial reddening of the mucosa of the upper lip, which may be intermittent or relapsing. The tongue is but slightly affected until the attack is well advanced. A reddening of the mucosa of the cheeks or of the floor of the mouth is followed by the development of irregular patches of superficial necrosis within the reddened areas. Necrotic patches appear also on the base of the tongue, soft palate, fauces, and gums. A fetid odor and increase in buccal secretion develops, with drooling of an egg white sort of secretion. The mouth becomes very inflamed, and the dog puts his muzzle into water as if it desired to drink, but makes no effort to do so. Vomiting results if forced feeding is attempted. Hiccoughs are sometimes observed.

The temperature is but slightly raised until shortly before death. Constipation is followed by diarrhea and even bloody discharges. An itching or burning sensation causes scratching or biting, producing a self-inflicted dermatitis. There is an eruption on the scrotum. The attack is usually acute and rapid, the dog dying within ten days.

"Black tongue" is prevented or cured by autoclaved yeast, and also by a water extract of autoclaved yeast. The curative principle is adsorbed by fullers' earth. Goldberger designated this principle P-P (pellagra-preventive) but it is now known as vitamin G. Dogs developed symptoms of polyneuritis when fed the experimental diet containing the heated yeast which protected them against "black tongue."

Mendel and Underhill's Studies on "Black Tongue."—Underhill and Mendel (1925) concluded that a condition which they produced in dogs by a diet similar in properties to that used by Goldberger, is similar to or identical with "black tongue," and that it is associated with a lack of some substance contained in butter fat, but different from vitamin A. The substance is present to some extent in meat and also in casein which has been boiled with alcohol. There is a seasonal variation in the curative value of butter, and its value decreases with age. Its potency appeared to be associated with its yellowness. Other foods rich in yellow pigments are particularly effective in alleviating the Chittenden-Underhill syndrome in dogs. They concluded that the curative principle is either a natural pigment or some unidentified substance associated therewith.

In a later study (1928) Underhill and Mendel report that replacing 2.7 per cent. of peas (in the peas, cracker meal, and cottonseed oil diet) by meat failed to protect the dogs. Bone ash plus a considerable amount of meat likewise was not protective. Even replacement of the peas entirely by raw lean meat did not protect. Raw pig liver (2.8 per cent.) prolonged life, but when dried at low temperature it was not effective. Frozen pig liver in abundance prolonged life. Butter fat 1.7 per cent. or cottonseed oil 3.3 per cent. did not prevent the disease. Replacement of the peas by meat, and of cotton seed oil by butter, caused the dogs to remain in much better condition. Cod liver oil was given to supply vitamin A. No benefit was afforded by feeding yeast. Fresh butter protected against the disease, but butter which had been stored for 11 months did not. Butter which had lost its yellow color was not effective. Egg yolk was curative.

One hundred and fifty grams of boiled, unpeeled carrots a day cured the disease and maintained the dogs in excellent condition. Melted lard was mixed with fresh carrots and allowed to stand 8 days, after which it was extracted with cold ether. The ether was evaporated, and the fat, which was bright yellow from extracted pigment, cured the disease in dogs. Furthermore, crystalline carotin prepared from carrots cured the disease; as little as 5 mg. per day was effective. Carotin is a highly unsaturated hydrocarbon ($C_{40}H_{56}$).

These experimental results come as a surprise after the studies reported by Palmer, who reared chicks on a nearly pigment-free diet (p. 181), and secured eggs from nearly pigment-free birds. It may be, however, that mammals, or at least certain species, require the yellow plant pigment carotin for the maintenance of normal metabolism.

Presumably the condition in dogs in the experiments of Mendel and Underhill, and in those of Goldberger, is the same, and its similarity to pellagra is unquestioned. It is most remarkable, therefore, that these distinguished workers should have obtained results and reached conclusions so diametrically opposed. All workers would probably agree that the anti-pellagra substance (vitamin G) is essentially lacking in corn meal, white flour, rice, and cottonseed oil, and probably other vegetable oils. Goldberger listed yeast, lean meat, milk, and eggs as protective or curative in the order named. He asserted that there is reason to believe that all vegetables—potatoes, turnips, string beans, tomatoes, cabbage, collards, turnip greens, spinach, etc.—and fruits contain the pellagra-preventive vitamin, but in small amounts. He found (1928) butter to be of little value. On the other hand, the Yale investigators obtained negative results with yeast, and found lean meat to be of low value, whereas yellow butter, yellow carrots, and the pure pigment carotin were very effective in curing the disease. Goldberger obtained little or no benefit with human patients given yellow pigmented vegetable foods or butter.

There are several assumptions in Goldberger's discussions, one or more of which must be unwarranted. He assumes that experimental "black tongue" is identical with the disease seen in dogs living under ordinary conditions in the South; that the disease which he produced experimentally is the same as that produced by Mendel and Underhill; that "black tongue" is pellagra in the dog. None of these views are fully established.

Out of the numerous confusing studies of the etiology of pellagra one can safely conclude that the disease is caused by a faulty diet. The evidence that it is caused specifically by deficiency of the heat-stable substance vitamin G is hardly convincing enough to fully establish Goldberger's theory. The theory of Jobling that light sensitization plays a rôle in the etiology of pellagra deserves more attention than has been accorded it. The researches of the past decade are of the greatest importance, however, since it is now possible to prevent the disease by proper attention to the food supply.

CHAPTER XXIV

IODINE DEFICIENCY AND GOITER

Iodine was first used in the treatment of goiter by Coindet when he painted goiters with tincture of iodine in 1820. Bousingault stated in 1825 that "until now iodine is the only specific known for goiter." In 1850 Chatlin stated that goiter was due to a deficiency or iodine in air, water and food, but he could not establish his view. Saint-Lager (1867) controverted the iodine deficiency theory and to a great extent discredited it.

In 1895 Baumann discovered that the thyroid gland contains iodine. Marine and Williams (1908) studied the histological changes which take place in the formation of goiter in animals. A fall in the iodine store always precedes any cellular changes; then comes a decrease in the chromatic colloid, followed by change in shape, together with multiplication of cells and glandular hypertrophy. They also demonstrated that pups born of a dog with three-fourths of the thyroid removed, the mother being kept on a diet low in iodine, had enlarged thyroids, with the same histological changes seen in goiter. Their thyroids had a low iodine content. The same dog was fed iodine during a later gestation and at birth the pups had thyroids normal in size, structure, and iodine content.

Iodine as Limiting Factor in Nutrition.—The maximum iodine content of normal thyroids is about 5 mg. per gm., with an average of 2 mg. per gm., and a total of 40 mg. for the normal adult human thyroid. As long as the iodine content remains above 1 mg. per gram of dried tissue, no cellular changes result, but changes are noted when the content falls below 1 mg. per gm. These data show that to function normally the thyroid must contain iodine within certain limits. There is danger in certain parts of the United States and elsewhere that human beings and animals may fail to secure in their food a sufficient amount of iodine. Iodine is essential for the formation of an organic iodine-containing compound which is a hormone or regulator of metabolism.

Isolation of Thyroxin.—Kendall (1915) isolated the active principle from the thyroid glands of animals and named it thyroxin. A lack of iodine in the diet prevents the formation of this hormone and leads to hyperplasia and enlargement of the gland. Kendall believed that thyroxin was a tri-iodo indole derivative, but Harrington succeeded in synthesizing the substance. It has the following structure:

Administration of 14 mg. of the synthetic product to two myxedematous patients in three doses over a period of 6 days caused a rise in the basal metabolic rate from minus 45 per cent. to plus 3 per cent. in one case, and from minus 32 per cent. to minus 6 per cent. in the other. In both cases the expected increases in pulse rate and loss of weight were also observed.

Goiter in Fish Hatcheries.—Salmon and trout, reared under crowded hatchery conditions, when fed liver and kept in water which is not changed frequently enough, have suffered greatly from goiter. It was shown by Marine and Lenhart (1910) that addition of potassium iodide to the water in which fish are kept causes retrogression of the thyroid and a return to normal. Fish fed hashed sea fish also showed improvement of their thyroids, presumably because sea fish contain iodine. Wild fish seldom have goiter although pike and bass in Lake Erie frequently suffer from it. Marine (1913) demonstrated in the fish hatcheries of Shady Grove, Pa., that the addition of an exceedingly small amount of iodine to the water controlled what had hitherto been a serious endemic goiter problem. This practice still prevails in fish hatcheries.

Endemic Goiter Regions.—As early as 1870 the farmers of Michigan were preventing endemic goiter and cretinism in sheep by the use of certain crude salt, but did not appreciate how the disease was prevented. In the Yellowstone River Valley there has been for some time an annual loss of enormous numbers of young pigs, lambs, calves, and colts as the result of thyroid disease. This most serious problem in animal production was studied by Smith (1917), who found that it was only necessary to administer sodium or potassium iodide to the pregnant animals in order to prevent the condition. Because of shortage of iodine, many of the pigs were born dead, others died within an hour or

two, while still others lived 24 to 36 hours. These pigs were born in a hairless condition, and with great enlargement of the thyroid gland. Hart and Steenbock (1918) have also observed this condition in pigs.

The most noted of the endemic goiter districts are (1) the Alpine mountain region of Southern Europe, including all Switzerland, Northern France, Northern Italy, and the Balkan States; (2) the Himalayan mountain region of Eastern and Southern Asia, the Gilget district of Northern India, and the plateau regions of Western China and Eastern Mongolia; (3) the Andes mountain region of South America, the most noted section of which is the Peruvian plateau; and (4) in North America, the entire basin of the Great Lakes, the basin of the St. Lawrence, the states of Colorado and Utah, and that portion of the United States and Canada known as the Pacific Northwest. In a well-known valley of British Columbia called Pemberton Meadows, the incidence of goiter was so great that it was difficult to raise calves, lambs and pigs.

Keith (1924) studied the problem of goiter in the Pemberton Valley. Many of the men, almost every woman, and every child born in this district, had an enlarged thyroid. Pigs were born hairless, calves were goiterous, mares became goiterous, and many colts were carried beyond full term and showed traces of myxedema. The farm animals were more subject to goiter in late winter and early spring. Many of the eggs of fowls, although fertile, failed to hatch, the apparent reason being a thickening of the white envelope within the shell. As a result of giving iodine, most of these conditions have now disappeared. No cases of goiter could be found among the inhabitants of an Indian village located on the same site in this valley; they were here many years before white men settled here. The Indians and their pigs obtained sufficient iodine from the large amount of salmon annually consumed.

McCarrison's Views on Goiter.—McCarrison (1922) stated that simple goiter is a deficiency disease due to insufficient iodine for the needs of the thyroid gland, or more properly, of the needs of the body cells for thyroxin. He gave the following facts: (1) thyroid hyperplasia is readily produced by diets deficient in iodine; (2) iodine in minute doses will prevent and cure goiter when administered at the proper time and season. He stated further: "While theoretically goiter can, and sometimes does, arise in consequence of a deficient supply of iodine

FIG. 30.—A group of dead and hairless pigs. Note the thickness of the necks indicating a goiterous condition. The mother of these pigs had been reared on a high protein and low roughage ration consisting of 15 parts of alfalfa, 25 of corn, 25 of oats, 25 of middlings, and 10 of oil meal, and kept under strict confinement. She produced two litters of hairless pigs before being changed to a ration of 33 parts of clover, 33 parts of corn and 33 of oats, on which the above pigs were produced. (Courtesy of Professor E. B. Hart, Wisconsin Agricultural Experiment Station.)

in the food, it arises more commonly in spite of the fact that there is present in the food a sufficiency of iodine. There is abundant proof of this statement; it will suffice, however, to mention in this connection the results of one fundamental experiment. If animals be confined to dirty cages they will develop goiter spontaneously, the size of the goiter being dependent on the season of the year, and the duration of exposure to the unhygienic conditions. If, however, they be confined in scrupulously clean cages of the same dimensions goiter will not arise. Since the food supply, and therefore the iodine supply, was the same in both cases, it follows that there are factors provided

Fig. 31.—The same sow that produced the pigs shown in Fig. 30. On the same ration —but fortified with 10 grains of potassium iodid per 100 lbs. of feed a normal litter was produced. (Courtesy of Professor E. B. Hart, Wisconsin Agricultural Experiment Station.)

by unhygienic conditions of life, as in dirty animal cages, which render the available iodine in the food insufficient for the needs of the body in these particular circumstances. A sufficiency may become an insufficiency in the presence of such unhygienic conditions. Further, if an additional supply of iodine be provided, or cod liver oil be administered, goiter will not develop in animals confined in dirty cages." He stated that previous work had demonstrated the importance of gastro-intestinal infection (in which may be included infection of the mouth, nose, tonsils, etc.) as a goiter-producing influence. He concludes the chief known factors, viz., physiological, nutritional, metabolic, toxic, infectious and nervous, may, in varying combinations,

cause goiter; and while, therefore, simple goiter is due theoretically to insufficiency of iodine, it is due in practice to a combination of factors which bring out this insufficiency.

Iodine in Drinking Water.—McClendon and Williams (1923) compared the iodine content of drinking water and the distribution of goiter throughout the United States. Division of the country into four zones showed 0.01-0.1 parts per billion of iodine with a rate of 15 to 30 cases of goiter per 1000. With 0.015-1.2 parts per billion of iodine in the water there was a rate of 5 to 15 cases per 1000. With 0.06-9.0 parts per billion a 1 to 5 rate

Fig. 32.—Stillborn goitrous calf. (Courtesy of Professor J. W. Kalkus, Western Washington Experiment Station.)

existed. With 1.4-10.0 parts of iodine per billion there was a rate of 0 to 1 per 1000.

They (1923) produced enlarged thyroids in rats by a diet very low in iodine. The controls were given one day a week drinking water containing 0.1 per cent. of iodine. At the end of 3 months the thyroid gland of the controls weighed from one-half to two-thirds as much as those not receiving iodine.

Little (1923) discusses the iodine treatment of water supplies for the prevention of goiter. The Water Bureau and Health Bureau of Rochester, N. Y., prescribed 0.0001 gm. of iodine taken daily over a period of 15-20 days as a preventive of goiter. Sixteen pounds of sodium iodide added to the city water supply every day increases the iodine content from one part to twenty

parts per billion. This treatment is reported to have given good results.

Ellms (1924) criticizes the treatment of city water supplies with iodine. He points out that it is very expensive and on the average only about 40 per cent. efficient. Chlorine applied to the water would tend to liberate iodine from the sodium iodide, and the iodine would tend to react with other constituents and possibly affect its therapeutic value.

Treatment of Goiter in School Children.—Marine and Kimball (1917) studied the effects of administering sodium iodide on the incidence and course of goiters in girls in the public schools of Akron, Ohio. In a complete census of the condition of the thyroid gland in the girls from the fifth to the twelfth grades of the school population in this goiterous district, it was found that 1,688 or 43.59 per cent. had normal thyroids, 2,184 or 56.41 per cent. had enlarged thyroids, and 594 or 13.4 per cent. had well-defined, persistent thyroglossal stalks. They administered sodium iodide to a large number of these girls, and 6 months later a reëxamination of their thyroids was made. The following table shows these results and also the data secured with another group of girls who did not take the prophylactic treatment.

TABLE XXX

Pupils Taking Prophylactic Treatment	*Pupils*	*Per Cent*	*Pupils Not Taking Prophylactic Treatment*	
			Pupils	*Per Cent*
Thyroids remained normal	283	100.0	637	74.0
Increased from normal to slight goiter	0	0.0	259	26.0
Small goiters (unaltered)	287	66.0	759	87.0
Small goiters (disappeared)	141	33.5	10	1.2
Small goiters (increased)	2	0.5	103	11.8
Large goiters (unaltered)	34	66.7	106	95.5
Large goiters (decreased)	17	33.3	5	4.5
Total	764		1,879	
Total number of girls examined	4,415			

These results indicate clearly the value of the administration of suitable doses of iodide as a means of preventing the hyperplasia of the thyroid gland.

Kimball (1923) found goiter to be at least 6 times more frequent in girls than in boys. He states that a large majority of goiters in boys are congenital and that this can be prevented by the administration of iodine to the mother during pregnancy. While occasionally a boy does develop goiter at puberty, or after illness, or as the result of some chronic infection, in most cases the goiter will disappear if it is treated promptly and properly. He believes the maximum good from a minimum of effort would result by applying the principle of goiter prevention to girls as a public health measure and that it can best be done through the public school organization. Education would insure the application of this method in later life.

Olin (1924) made a detailed study of iodine deficiency and the prevalence of goiter in Michigan. In four counties 31,612 children were examined. Of this number 14,914 had goiters; 40.5 per cent. in boys and 53.8 per cent. in girls. It was proposed that by law all salt sold for human and animal consumption in goiterous regions be required to contain sufficient iodine so that the minimum requirement of 300 mg. per year would be normally taken by everyone. Dubois (1924) reported a survey of 1805 persons of different ages at Alma, Michigan, for goiter. Of 1400 grade and high school children, 40 per cent. were goiterous; of these, 41 per cent. were boys and 59 per cent. girls. In the college, 38 per cent. were goiterous (44 per cent. boys; 56 per cent. girls). Wallace reports examination of 69,256 pupils in Utah; he found 31.4 per cent. of boys and 54.2 per cent. of girls were affected.

Kimball (1928) gives a record of many significant community studies in the prevention of goiter by providing iodine in the form of either tablets or iodized salt. Where iodized salt is used, one part of sodium or potassium iodide is incorporated in 5000 parts of table salt. Such salt has been widely used in goiterous districts. In Wexford County, Michigan, iodized salt was introduced in October, 1925; in 18 months the incidence of goiter had decreased 25 per cent.

McClendon (1924) has refined methods to determine very small amounts of iodine and has studied the iodine in foods and water in many places. The water of Lake Superior contains approximately 1 part of iodine in 100 billion parts of water, and

that of the lower Mississippi valley contains 10,000 to 15,000 parts of iodine to 100 billion parts of water. Some of the deep wells of Texas show more than 18,000 parts.

Vegetables and grains contain from 100 to 1000 times more iodine in goiter-free sections than in the Lake Superior region where goiter is endemic. According to McClendon, the intake of iodine in the food and drink of a normal young man was 0.057 mg. in 3 days; the amount excreted in this period was 0.021 mg. The actual daily requirement was 0.012 mg. of iodine.

McClendon estimates from the amount of iodine in the water and common foods in goiterous districts that it would require 10 years for a normal adult to accumulate 40 mg. from these natural sources. This is the average amount Marine found in normal adult thyroids.

In Charleston, W. Va., before 1900, goiter was exceedingly rare, but soon after this date it became common. At this time 60 per cent. of school girls at Charleston and Huntington had enlarged thyroids. A study showed no significant change had occurred in food or water, but that the source of table salt was new. Until 1900 the table salt they used came from local salt wells, and was described by older citizens as "coarse and containing dirty brown particles." In 1898 refined salt came into use in this district, which thereupon became goiterous.

Many medical men have viewed with alarm the introduction of iodized salt for household use, and it was predicted by some in 1916 that this would result in epidemics of exophthalmic goiter and an increase in toxic adenomatous goiter.

Kimball (1928) after years of practical experience in the study of goiter in the Great Lakes region states: "We have never seen an authentic case of hyperthyroidism in children that could by any stretch of the imagination be attributed to the prophylaxis of goiter through the schools." He administered in Akron, Ohio, 2 gm. of sodium iodide over a period of two weeks to a child, and gave more than ten thousand treatments; the total of bad results was 10 cases of mild iodide rash with no symptoms of hyperthyroidism.

Kimball emphasizes the importance of prevention of thyroid injury during pregnancy, and states that we cannot stress too strongly the importance in endemic districts of assisting all prospective mothers by encouraging the general use of iodized salt during pregnancy. He believes one or two iodostarin tablets a week throughout pregnancy and lactation would be excellent.

He states that one hundred years' experience shows that the treatment of goiter is unsatisfactory and accomplishes little toward the control of the disease,—and yet of all diseases endemic goiter is probably more easily prevented than any other. Most children in endemic goiterous areas are born with injured thyroids; therefore the prevention of goiter is in great measure a pre-natal problem.

It appears that a lack of iodine leads to a lack of thyroxin, the active principle of the thyroid, because of a lack of the wherewithal to make it. Lack of thyroxin causes enlargement of the thyroid gland in response to under-supply of this substance. When the gland has not enlarged, but is starved for iodine, the provision of iodine will prevent goiter. When the gland has already enlarged, the giving of iodine may result in the formation of more thyroxin than is necessary, at least for a time. At any rate the gland is stimulated by iodine to produce thyroxin, and so is kept active. It would seem more logical under such conditions to provide thyroxin rather than iodine, for this would rest the gland rather than stimulate functional activity. When there is hyperfunctioning of the thyroid, the provision of liberal amounts of iodine may do distinct harm because of over-production of the hormone, thyroxin, which stimulates metabolism to a remarkable degree. It will be apparent, therefore, that the goiter problem is not so simple as many believe it to be. Persons with goiters should consult a specialist and be guided by his counsel.

CHAPTER XXV

SPECIFIC EFFECTS OF FOODS IN BLOOD REGENERATION

The average life of the red cell of human blood is 30 days, and 3 to 5 per cent. of the red blood corpuscles are destroyed daily. The blood may be impoverished through failure of the body to produce new red cells as fast as they disappear. Such a situation arises when the food is deficient in the substances which are necessary for the formation of either the stroma or the hemoglobin of which the corpuscles are principally composed. Impoverishment may also result from hemorrhage, infection or infestation of the intestine by ameba, hookworm, or fish tapeworm; or from poisoning from hydrogen sulfide, lead, mercury, or arsenic. Secondary anemia generally, if not always, accompanies rickets, osteomalacia, infantile tetany, xerophthalmia, simple goiter, scurvy, pellagra, and sprue, as well as various metabolic diseases involving the liver, kidney, spleen, pancreas, and endocrine glands, and in persons with malignant neoplasm. It has long been known that when the diet is satisfactory, anemia from any of the above causes tends to disappear within a few weeks or months when the cause is removed; in other words, a cure is effected. In pernicious anemia, on the other hand, improvement of the blood can be brought about by the provision in the diet of a special principle which is abundant only in liver and kidney. The normality of the blood is maintained only so long as this principle is provided,—hence no cure is effected, but only an amelioration which, however, may be maintained indefinitely by adherence to a suitable dietary.

The Diet in Relation to Blood Regeneration in the Normal Body.—Whipple and Robscheit-Robbins and their co-workers (1925-1928) studied the effects of different food-stuffs on blood regeneration in normal dogs made anemic by repeated bleeding. They showed that during fasting periods after hemorrhage the dog can regenerate hemoglobin in considerable amount in excess of the daily wastage. During periods when dogs were fed only

sugar, the hemoglobin formation was distinctly less than during similar fasting periods. Under fasting conditions body tissues are broken down in order to keep up repair of waste in the organs most essential to life, and this katabolism furnishes the materials for corpuscle construction. Carbohydrate feeding exerts a sparing action on body proteins at source and limits the availability of cleavage products which can be recast into hemoglobin. Gliadin fed as the sole source of protein does not modify hemoglobin formation because it is deficient in several amino-acids, i.e., it is an incomplete protein.

These investigators report that a diet of white bread and skim milk may cause a slow gain in blood pigment from week to week and may result in complete blood regeneration, but is barely sufficient for this purpose. Cracker meal (a mixture of wheat flour, barley flour and rice flour) with milk or lard or butter caused blood regeneration at a rate similar to bread and milk alone. Rice, potatoes and skim milk gave similar results. Casein alone is somewhat more efficient than gliadin alone, but is a poor pabulum for hemoglobin formation. Cooked beef heart was very effective, but cooked liver was remarkable in that it caused rapid blood regeneration even under the most unfavorable dietary conditions. As early as 1920 Whipple and Robscheit-Robbins found kidney essentially the equal of liver for this purpose.

Conditions Essential to Iron Assimilation.—For many years iron has been administered as a remedy for anemia, since hemoglobin is rich in iron. The results were sufficiently gratifying to prevent until recently the discovery that even when iron is provided in abundance in assimilable form it cannot be synthesized into the respiratory pigment hemoglobin unless the body is furnished a certain amount of copper. Hart, Steenbock, Waddell and Elvehjem (1928) found it possible to produce anemia in young rats of 3-4 weeks of age by restricting them to a diet of whole cow's milk. On this they grew well for 4-6 weeks, when their weight became stationary and soon a rapid decline set in. Blood examination showed erythrocyte counts of 2.87 to 6.95 millions per cmm., and hemoglobin values of 5.1 to 8.9. Normal values for the rat are, erythrocytes six million to seven million per cmm., and hemoglobin 10.8 to 12.6 gm. per 100 c.c. of blood.

When iron salts (chloride, sulfate, acetate, citrate or phosphate) were fed with the whole milk diet in amount to provide

0.5 mg. of iron daily six days per week, the condition of the blood was not improved. When the ash of lettuce, beef liver or yellow corn was fed, in addition to the iron and milk, the anemia was rapidly relieved. It thus became evident that the ashes of these substances contained some inorganic constituent which is essential for the utilization of iron for hemoglobin formation. It was next shown that this substance is precipitated by hydrogen sulfide in acid solution. A test of the effect of feeding both a copper and an iron salt with milk showed that copper is the element which was present in liver, lettuce, etc., which enabled the animals to utilize iron. It is interesting to note in this connection that Mallory (1927) reports that copper in any form is easily absorbed and that it injures red blood corpuscles and causes hemolysis.

Manganese and Iron Assimilation.—Titus, Cave and Hughes (1928) have brought forward evidence that manganese is also an essential element in mammalian nutrition and that it is associated with iron assimilation. Drabkin and Waggoner (1929), in a most interesting paper on hemoglobin maintenance upon synthetic diets, found that the hemoglobin maintenance levels of dogs and the speed of recovery after single large bleedings were not influenced by the addition of $CuSO_4$ to the diet when 1.3 mg. of copper per kilogram of body weight was fed. They also found that the growth, general appearance, and hemoglobin maintenance were normal in rats on a copper-containing and a copper-free diet. Five rats which had been made anemic on a whole milk diet were promptly cured when they were changed to the copper-free synthetic ration. It is clear from these experiments that more work must be done to establish under what conditions iron is assimilated.

Comparative Values of Foods for Blood Regeneration.—Whipple (1928) has published the following tables which show the comparative value of a number of foods for blood regeneration.

TABLE XXXI

Hemoglobin Production Influenced by Diet

Diet, Grams Daily	*Hemoglobin Production per Two Weeks' Period* Gm.
Bread, 450	3
Bread, 350	3
Milk, 450; bread, 450	3
Milk, 450; bread, 350	3

TABLE XXXI—*Continued*

HEMOGLOBIN PRODUCTION INFLUENCED BY DIET

Diet, Grams Daily	*Hemoglobin Production per Two Weeks' Period* Gm.
Cream, 100; bread, 350	12
Cream, 100; bread, 500	10
Butter, 100; bread, 350	10
Butter, 75; bread, 450	11
Cheese, 100; bread, 450	20
Beef muscle, 200; bread, 400	25
Pig muscle, 300; bread, 300	20
Heart muscle, 200; bread, 300	28
Codfish, 100; bread, 300	9
Fresh fish, 250; bread, 300	10
Spinach, 250; bread, 400	14
Greens, 200; bread, 300	20

TABLE XXXII

HEMOGLOBIN PRODUCTION INFLUENCED BY DIET

Diet, Grams Daily	*Hemoglobin Production per Two Weeks' Period* Gm.
Beef liver, 400	100
Beef liver, 300; bread, 300	50
Chicken liver, 200; bread, 250	75
Fish liver, 150; bread, 350	0
Beef kidney, 250; bread, 250	90
Pig kidney, 200; bread, 350	70
Chicken gizzard, 300; bread, 200	85
Bone marrow, powdered, 30; bread, 600	25
Beef spleen, 200; bread, 250	25
Brains, 300; bread, 300	25
Pig pancreas, 250; bread, 350	30
Apricots, 200; bread, 300	40
Peaches, 200; bread, 300	40
Raisins, 300; bread, 350	25
Apples, 250; bread, 350	35
Prunes, 250; bread, 350	35
Raspberries, 250; bread, 350	0

The high value of peaches and apricots for blood regeneration is of special interest, since these fruits may serve a useful purpose in the diets of patients when the protein content must be kept low. Whipple has tested and recommends in the diet for anemias, all kinds of liver except fish, chicken gizzard, liver sausage, and blood sausage.

Pernicious Anemia.—There is now much reason to consider pernicious anemia to be a deficiency disease. There is no longer

any doubt that there exists an anti-pernicious anemia substance. A deficiency of any one of the known vitamins will produce the characteristic deficiency syndrome in any previously normal individual. Although most persons live habitually on diets containing an amount of the specific substance much smaller than is required to maintain a normal blood balance in a patient with pernicious anemia, they do not develop the disease. The conclusion is obvious that pernicious anemia is due not solely to shortage of a specific substance in the diet. There appears to be in persons who develop pernicious anemia while on a diet which does not produce the disease in most people, an internal obstacle to the assimilation of the specific substance, or else an exaggerated requirement for it. It appears that for the development of pernicious anemia there must be something wrong with the individual, either hereditary or acquired, in addition to inadequacy of the diet.

This specific substance is found most abundantly in liver (sheep, beef, pig, chicken) and in kidney. The results of Whipple and Robscheit-Robbins recorded above, which show such remarkable differences in the value of different foods for red cell production in secondary anemia, suggest that the substance may exist in plants,—but this has not been proven by any recorded observations on pernicious anemia patients. It is not known whether it can be produced by the body under normal conditions; if it is, one may visualize how the food may supply sufficient precursors for its formation, yet synthesis may not be accomplished because of defect in the tissue which normally produces it. The provision of the specific substance by feeding liver would then cause recovery in a manner analogous to the restoration of the cretin by thyroid feeding. The abundance of the specific substance in kidney as well as in liver is confusing to this view, yet it may be that the kidney tissues have a special capacity to adsorb it.

Koessler's Studies on Experimental Pernicious Anemia.—Pernicious anemia always follows a prolonged history of digestive disturbances and is preceded by achylia gastrica and eventually achlorhydria. Koessler (1926) conjectured that failure of hydrochloric acid production by the stomach might be sufficient to initiate a series of events which would terminate in pernicious anemia. Deficiency of vitamin A is known to cause injury to epithelial cells generally, and to reduce or obliterate their secretory power (tear glands, salivary glands, etc.).

Achlorhydria prevents peptic digestion, and permits bacterial cleavage of proteins in the stomach. Periodic inundation of the duodenum with undigested and bacterially contaminated protein material results in impaired and abnormal protein cleavage in the intestine and the production of excessive amounts of putrefactive substances, among which are the powerful pharmacological principles histamine and tyramine. Deficiency of vitamin A, according to their view, perverts the functions of the intestinal mucosa in respect to secretion of digestive fluids, absorption, and power to detoxicate the injurious amines. Since in achylia gastrica the entire small intestine may be the seat of putrefaction of protein, there are produced extraordinary amounts of amines which, on entering the blood injure the hemopoietic structures in the marrow. Acting on this conjecture, they produced in young rats deprived of vitamin A a condition of the blood remarkably similar to that seen in pernicious anemia. The diet consisted of bread 30, rice 20, corn meal 20, casein 12, minerals 5, cottonseed oil 12.5 per cent. together with brewer's yeast 0.1 gm. daily. Their conclusions were that blood regeneration cannot take place without vitamin A. The provision of vitamin A, after rats have been long depleted and have a blood picture presenting many of the features characteristic of pernicious anemia, brings about rapid formation of new red blood cells. The rate and intensity of blood regeneration was a function of the quantity of vitamin A administered. Cod liver oil and butter fat were used as sources of vitamin A.

From the results of Whipple, Minot, and others it is obvious that the specific substance in liver which influences pernicious anemia is not vitamin A. It is very probable that one or more unknown factors operated in the diets used by Koessler to produce the analogue of pernicious anemia in rats.

Koessler's emphasis upon the significance of perverted function of the stomach and intestinal mucosa with the attendant interference with digestion as a factor in the etiology of pernicious anemia, is most interesting in the light of the recent studies of Castle and Locke (1928). The latter investigators found a response similar to that attending the feeding of liver to patients with pernicious anemia could be secured by feeding skeletal muscle previously digested in a normal human stomach. Muscle meat was without effect unless given this treatment. The results of their study suggest that the deficiency of the specific substance in pernicious anemia depends upon loss of digestive capac-

ity. Heath (1928) reports 33 cases of pernicious anemia patients who were retested from two to eighteen months after remission due to liver feeding, and in no case was free hydrochloric acid demonstrated in the stomach.

Chemical Studies on the Nature of the Specific Substance.—Cohn and co-workers (1928) find that the active principle in liver is soluble in water, and is precipitable by alcohol. It is insoluble in ether. Preparations free from iron and proteins, carbohydrates and lipoids were potent in relieving pernicious anemia. The substance is precipitated by phosphotungstic acid but not by basic lead acetate. The material precipitated by phosphotungstic acid contained about 19 per cent. of nitrogen, and is believed to be a nitrogenous base or a polypeptide, of which but 0.6 gm. per day sufficed to produced a pronounced reticulate (young red corpuscle) rise in the peripheral blood in pernicious anemia patients.

West and Nichols (1928) have fractionated with silver precipitation the material precipitated from liver extract by phosphotungstic acid. Silver does not precipitate the specific substance. On reprecipitation of the filtrate from the silver precipitate with phosphotungstic acid and decomposing the latter precipitate, a preparation was obtained which contained 12-14 per cent. of nitrogen and 20 per cent. of amino nitrogen, which increased to 40 per cent. on hydrolysis. The concentrate was free from phosphorus and iron, and contained but a trace of sulphur. Copper was probably absent. Organic tests gave the following reactions: biuret, +; diazo, +; Hopkins-Cole, weak+, naphthol test for arginine, +; polariscopic readings gave a weak levorotation.

The above described research on the conditions under which impoverishment of blood may take place and the necessary nutritive conditions under which blood regeneration is possible, constitute one of the most spectacular features of modern nutrition studies. Although much remains to be done in this field, the work already accomplished is of inestimable value to the clinician.

CHAPTER XXVI

THE NURSING MOTHER AS A FACTOR OF SAFETY IN THE NUTRITION OF THE SUCKLING

Diet During Pregnancy and Lactation.—The periods of pregnancy and of lactation are of special significance in the lives of both mother and young. Researches relating to the effects of experimental diets on the pregnant and the nursing mother are therefore of special interest, as are also those observations which show the effects on the mother and child under different conditions of life where there are marked contrasts in the quality of the food supply. These topics will form the subject of the present chapter.

Since this book deals with principles rather than with practical dietetics, the management of the diet of the pregnant and nursing mother will not be considered in detail. Since it is of the greatest importance that the expectant and nursing mother should be under the supervision of a physician, it is best to leave the planning of her dietary to him. Day (1923) and Mendenhall (1924) have discussed in detail protective feeding during pregnancy.

Osteomalacia in Women.—The observation of Maxwell (p. 284) on the widespread occurrence of osteomalacia among women in Northern China and Southern Manchuria already described, illustrates that safeguarding the calcium metabolism during pregnancy may become a problem of the first order in public health work. The frequent occurrence of rickets during the nursing period emphasizes the fact, not generally appreciated, that breast feeding has its limitations in safeguarding the infant's health, and that proper feeding of the mother at this time is as important as breast feeding itself.

Newborn Young Dependent on Milk.—For a time after birth every mammal is incapable of taking the type of diet which suffices for the nutrition of the adult. It must have milk for a certain period, although the duration of dependency is not the

same for all species. The guinea pig appears to have the shortest period of dependence upon the mother; it is born in a very advanced state of maturity and can eat grass or succulent vegetables the first or second day after birth. The rat is born in a very immature state; it does not open its eyes until the fourteenth to the sixteenth day. It may be weaned at the age of 25 days provided the diet of the nursing mother is satisfactory. If the mother's diet has been faulty in any respect it may be necessary to prolong the nursing period, at times to 50 days, during which time the young supplement their milk diet with the diet of the mother. The young pig (swine) when six to eight weeks of age eats fairly liberally of forage plants, cereals, and roots.

During the first year of life the human infant must live on a diet consisting in great measure of milk. It cannot thrive in an optimum manner without a fairly liberal supply of milk during the early years of childhood. It seems certain that a common error in America at the present time is a too liberal substitution of other foods for milk during the first years of childhood.

Milk Secretion as Related to Diet.—Babcock (1905) deprived cows of sodium chloride, other than that contained in their food, for 15 months. He noted no decrease in the milk yield until the animals began to fail rapidly. The fat content of the milk was slightly higher than in the control group. Eccles and Palmer (1916) fed cows super-abundant rations and also studied the effects of under-feeding. They conclude that secretion of milk is controlled by at least two factors: (a) chemical, and (b) nervous. The chemical stimulus is the predominating one immediately following parturition; it is this stimulus that fixes the maximum flow for each individual animal. Just what controls this stimulus they are unable to say, but they believe it depends upon heredity as well as physiology.

Their experiments with super-feeding showed that cows are unable to increase their milk flow beyond the fixed stimulus for this function in the individual. A high-class dairy cow is one born with a strong inherent tendency to secrete milk, and the general problem in feeding is to supply sufficient nutrients to support the milk production which she has inherited. Several factors influenced the effects of under-feeding, viz., the stage of lactation, the degree of under-feeding, the character of the ration, the state of flesh of the animal, the plane of nutrition

of the animal previous to under-feeding, and the length of under-feeding.

Meigs (1922) has given an excellent review of the early studies of animal husbandry investigations relative to the effect of changing various components of the diet on the quality of the milk. He points out that many experiments were poorly planned and controlled, but there is, nevertheless, an agreement that the quantity of the milk yielded is highly dependent on the quantity of the protein supplied in the food.

Faulty Diet and Milk Production.—Since many laboratory experiments had demonstrated that young rats could not synthesize vitamins A and B, but must derive them from their diet before growth could take place, the question arose as to whether or not the mammary gland could synthesize these essentials for the nursing young when the diet of the mother did not contain them. In order to discover whether the mammary gland could synthesize vitamins A and B, McCollum and Simmonds (1916, 1918) carried out a series of experiments with lactating rats whose diets were faulty in known respects. The rats were kept on a diet of natural food-stuffs until the young were born. After the young were delivered, the litters were reduced to four in order that the nutritive undertaking of the mother should not be burdensome. Several series of experiments were conducted in which the basal ration consisted of purified casein, dextrin, inorganic salts, and agar-agar. This ration is adequate for growth in the rat except for the absence of vitamins A, B and D. In a second series, the same ration was used, except that butter fat was added to furnish vitamin A and a little D; a third series receive vitamin B in the form of an alcoholic extract of wheat germ, but no A was supplied. The control group had vitamins A and B present in the diet in the form of butter fat and an alcoholic extract of wheat germ. The results showed conclusively that vitamins A and B pass into the mother's milk only when they are present in the diet of the mother.

Another series of experiments with nursing rats was designed to show to what extent the mother could supplement in milk synthesis an inadequate diet through the agency of the mammary gland. Rats were kept on an adequate diet until they delivered their young. Each litter was reduced to four. Diets faulty in known respects but derived from natural foods were given the nursing mothers instead of the purified diets discussed above. The experiments demonstrated the following facts: (a) that the

mother rat could take a single seed, such as wheat, corn or rolled oats, and produce milk in sufficient quantity and of such quality that growth could proceed at a subnormal rate, whereas the young themselves could not grow at all on the mother's diet; (b) when the inorganic deficiencies of the seed are corrected, the mother can induce much greater growth in the young than without such correction (rat 1019, Chart IX, page 431); (c) the improvement of either the protein or vitamin A content of the seed causes little improvement in the quality of the milk when the mineral content of the diet remains uncorrected. It had been demonstrated that young rats cannot grow on a diet of cereal grains, and that the first limiting factor is the mineral content. The nursing mother can take such a diet and put into the milk for a limited time an inorganic salt content which is much more satisfactory for the nutrition of the young than is contained in the seeds composing her diet.

The question may be raised as to whether the rats were producing milk on these faulty diets. The observation of Decaisne (1871) that during the siege of Paris young and vigorous women were able to produce enough milk to maintain their infants, bears on this point. In some instances the mothers, while partially fasting, increased their infants' weight.

Vitamin Content of Milk Dependent upon Mother's Diet.— Andrews (1912) attempted to throw light on the etiology of infantile beri-beri by inducing Filipino women, whose infants had died of this disease, to nurse young pups. All of these pups developed paralysis of the posterior extremities, a characteristic symptom of the disease. Our experiments with rats confined to a diet free from vitamin B confirm and explain Andrews' results. They establish the fact that milk production can take place under dietary conditions such that the product is deficient in certain complexes necessary for growth. Dr. E. Roxas of the Philippine Islands and Dr. A. do Amaral of Brazil (personal communications) inform us that mothers suffering from beri-beri produced considerable quantities of milk. Kennedy and Dutcher (1922) also found that the diet determines the amount of vitamins A and B in the milk. Kennedy, Palmer and Schlutz (1923) found breast milks to vary greatly in their content of B. Ten c.c. of a breast milk as a rule was not so potent as a source of this vitamin as 10 c.c. of cow's milk. Their results indicate that vitamin A in human milks is as a rule more satisfactory than the B content. The milk of a mother whose diet was

deficient in A and B produced milk which was deficient in these when it was tested on rats. Since at the time these studies were conducted vitamin G was not known, it is impossible on the basis of existing data to state more than the established fact that milk contains G and that it is in all probability derived from the food.

Nelson, Lamb and Heller (1922) demonstrated that young rabbits nursing a mother on a diet deficient in vitamin A developed xerophthalmia. The eyes of the nursing young became clear and bright when butter fat was added to the mother's diet.

Macy, Outhouse and co-workers (1928) have made a very critical study of the vitamin A and B content of human milks. They give a detailed account of their technique, which can be duplicated in any laboratory. Their studies are the most complete yet reported on this problem. The following discussion is taken from their papers.

The Quantitative Estimation of Vitamin A.—Milk from women on the average American dietary is apparently a rich source of vitamin A, since 2.5 to 3 c.c. of the mixed milk from a group of wet nurses in various stages of lactation were sufficient to satisfy the nutritive demands for this factor during growth and reproduction in the rat. However, with this daily allowance, the vitamin A content of the diet is too low for the increased metabolic demands accompanying lactation, as evidenced by a slight subnormality in the rats at weaning age and in the low amount of vitamin A the mother was able to confer to the tissues of her 6 young, although she appeared to be in good physical condition. Cases were observed in which such young rats developed xerophthalmia within 6 to 12 days after being placed on the standard vitamin A-free ration.

The Quantitative Estimation of Vitamin B.—The anti-neuritic potency of mixed human milk is very slight. As much as 25 to 35 c.c. is required daily for normal growth in the rat, but even these amounts are insufficient to satisfy all the demands of the mother and her young during lactation and the rapid growth period of the young. These data demonstrate that food materials rich in this important food component should form a prominent part in the diet of pregnant and lactating women. Food substances rich in vitamins, furthermore, should be introduced early in the nursing period of the infant, whether it be breast- or artificially-fed.

Vitamin A and B Content of Cow's Milk.—A study of the vitamin A and B content of cow's milk was made under the identical experimental conditions maintained in the human milk studies. The milk represented a composite from 450 Holstein cows fed a monotonous unchanging standard daily ration, so that a possible seasonal variation due to a change in the food was therefore eliminated. Three c.c. of fresh raw cow's milk may contain adequate vitamin A to produce satisfactory growth in the rat, but this small quantity does not always protect against secondary pathological conditions; at autopsy, either single or double mastoid involvement was found in 77 per cent. of the rats which had received 3 c.c. of raw milk daily. The increment in weight was normal for 4 weeks upon the addition of 12 c.c. of milk to the standard vitamin B-free ration, for 8 weeks with 15 c.c. of milk, and for about 12 weeks with 20 c.c.

Vitamin B Requirement of Nursing Young.—Sure and Schilling (1928) found that nursing young rats suffering from deficiency of vitamin B die with accompanying hemorrhages in the osteogenetic tissues; hemorrhages were also found in certain of the viscera. The diets on which beri-beri or polyneuritis developed in the nursing young proved entirely satisfactory for maternal welfare and for the growth of non-lactating individuals. It was suggested that a deficiency of vitamin B in the food of infants in this country may be considerably more prevalent than has been recognized.

Vitamin D Content of Human Milk.—Kennedy and Palmer (1925) made a study of the value of human milk fat as a source of vitamin D. They found that at least 8 per cent. was necessary for the production of a distinct "line test" in rats made rachitic by the McCollum ration 3143. Outhouse, Macy and Brekke (1928) studied human milk as a source of this factor. They found human milk fed in amounts of 25, 30 or 40 c.c. daily contains no anti-rachitic factor. Under the same carefully controlled conditions 30 c.c. of cow's milk fed daily for 7 days induced marked healing of rachitic lesions in rats. It is clear from these studies that human milk is very low in its vitamin D content. This fact explains the occurrence of rickets in breast-fed infants.

Diet of Mother and Susceptibility of Young to Rickets.—Hess and co-workers (1923) found that cod liver oil or the vitamins contained in it were not transmitted through the mother's milk in sufficient amounts to afford protection to the

young, but the oil afforded great protection when fed during the pre-experimental period. They found that the young animal stored the protective substance derived from the food after it begins to depend upon the stock diet, but was not protected against rickets by giving cod liver oil to the mother during pregnancy.

McCollum and co-workers (1927) studied the problem of passage of vitamin D into the milk when cod liver oil was fed to the mother rat. When female rats are fed cod liver oil before mating, during pregnancy, and during the first two weeks of nursing their young are protected to a certain degree against rickets when they are later subjected to a rickets-producing diet. Their experiments showed that vitamin D of cod liver oil passes into the mother's milk.

They also reported that a satisfactory diet except that it was low in its vitamin D content when fed to female rats before mating, during pregnancy and during lactation, does not necessarily confer much protection on their young when these are later fed a rickets-producing diet. Their data suggest that it would be the part of wisdom to supplement the diet during pregnancy and lactation with a source of vitamin D. They did not determine whether sunshine or irradiated ergosterol could be used in place of cod liver oil.

Nutritive Value of Winter and of Summer Milks.—Dutcher and co-workers (1920) have shown that 20 c.c. of summer milk were superior in anti-scorbutic potency to 60 c.c. of winter milk. Their results showed that when a cow passed from green pasture to dry feed, her milk did not immediately undergo deterioration in respect to its anti-scorbutic value. Only after a period of 5 to 8 weeks was there sufficient change in the quality of the milk to show its effects on guinea pigs. On ingesting fresh food the milk immediately rose in its anti-scorbutic value.

Hart, Steenbock and Ellis (1920) showed that milk of cows which had been confined for a year to rations consisting of air-dried grains and forage plants, and which had never been allowed during these months to eat any fresh or green food, was very deficient in vitamin C. Their results show clearly that the content of the anti-scorbutic substance in milk is dependent upon the diet. Hughes and co-workers (1927) have recently expressed the opposite view.

Diet and Composition of Eggs.—A remarkable observation of made by Pollard and Carr (1924) is of interest in connection

with the possible variation in composition of a secretory product. They fed pigeons diets restricted to a single plant seed supplemented with grit, oyster shell, ground bone, salt, charcoal, and tap water. The birds were therefore restricted to the seed grain for protein and vitamins. They fed wheat, corn, oats, barley, rye, kafir, hemp, popcorn, sunflower seed, soy bean seed, field peas, and buckwheat. The birds were fed about 6 months before the samples of eggs were secured for analysis. Table XXXIII shows the remarkable differences in the chemical composition of the eggs secured from birds on the different diets.

It will be seen from the table that there is a wide difference in the per cent. of total nitrogen in the different lots of eggs; the highest, kafir, contains 1.6 per cent., and the lowest, oats, 1.24 per cent. The nitrogen distribution, or character of proteins, was quite different in the various lots. No ammonia nitrogen was found in the rye or field pea eggs, whereas those from kafir, hemp and barley contained nearly 2 per cent. Only the eggs which contained a high melanin (tryptophane) content were hatchable. The pigeons rated the grains in the following order of decreasing efficiency: wheat, rye, oats, corn, kafir, barley, field peas, hemp and soy beans, popcorn, buckwheat, and sunflower. The last three were insufficient for maintenance of weight and no eggs were produced from these lots. Kafir is comparable to rye in its egg-producing properties, but the eggs were abnormal in composition and none were hatchable. Usually four eggs were laid in place of two when kafir was fed.

The secretion of the material of the yolk and of the white by the ovary and oviduct respectively represent the elaboration of a highly organized system designed by nature for the development of a young bird. One would expect, from knowledge hitherto available, that if the entire list of substances necessary for the formation of the proteins and other complexes was not satisfactory, the bird would fail to lay eggs. It seems certain, from the above data, that eggs were produced which contained proteins of abnormal structure, and that a protein structure departing widely from the normal pattern was formed. In the light of such data it seems not improbable that the composition of milk proteins may differ to a very significant degree from the normal when the proteins of the lactating animals are peculiarly constituted.

Diet and Quality of Milk Produced.—McCollum and coworkers studied the effect on the rate of growth of young rats

TABLE XXXIII

THE NITROGEN DISTRIBUTION OF PIGEON EGGS FROM VARIOUS CEREAL GRAINS EXPRESSED IN PER CENT OF THE TOTAL NITROGEN OF THE EGGS

Ration	*Per cent. of N*	*Ammonia N*	*Melanin N*	*Amino N in bases*	*Non-amino N in bases*	*Amino N in filtrate of bases*	*Non-amino N in filtrate of bases*	*Total summation*
Rye	1.39	0.00	4.06	12.66	9.92	58.84	14.12	99.60
Rye	1.25	0.00	4.00	12.20	9.70	60.00	13.10	99.00
Kafir	1.54	1.76	1.92	14.12	22.48	53.94	4.20	98.42
Kafir	1.66	1.80	1.98	14.32	22.16	53.28	5.16	98.70
Wheat	1.42	0.71	3.90	19.16	13.00	60.44	0.72	97.93
Wheat	1.48	0.81	4.20	18.60	13.52	62.04	1.38	98.55
Oat	1.28	0.18	3.22	12.06	9.80	71.40	2.00	98.66
Oat	1.20	0.20	3.32	12.34	9.80	70.60	2.74	99.00
Hemp	1.43	2.11	1.00	11.74	9.54	67.40	7.10	97.78
Hemp	1.53	2.00	1.00	11.84	9.58	66.80	7.80	99.02
Canada field peas	1.24	0.00	1.33	11.00	18.97	.....	.....	.....
Canada field peas	1.36	0.00	1.50	10.40	18.93	.....	.....	.....
Barley	1.39	2.07	0.66	14.74	17.57	50.00	1.94	98.00
Barley	1.47	1.72	0.80	14.34	18.93	58.00	2.94	97.73
Corn	1.26	0.91	3.47	11.26	12.22	70.00	3.20	101.06
Corn	1.38	1.00	3.52	11.00	12.10	70.40	2.48	100.50

of diets of different types when fed to the lactating mother. The results are shown in Tables XXXIV, XXXVI and XXXVIII. In Table XXXVIII are shown the weights of litters of young containing different numbers and at various ages during the nursing period, as well as later, after they became able to supplement the mother's milk with her diet. These weights serve as controls for comparison of the weights of the young of other mothers whose diets were faulty in certain respects. Although the data are self-explanatory, a few cases of marked contrast will be mentioned.

One of the females had 5 young on diet 2153, which is a satisfactory diet. At 15 days of age the collective weight of the young was 137 gm. A rat on diet 2365 (containing 9 per cent. of protein, two-thirds derived from barley and one-third from navy beans) likewise had 5 young; when these young were 18 days of age they weighed collectively 70 gm. The protein moiety of this mother's diet was of rather poor quality and for this reason she was unable to secrete a satisfactory milk supply. The tables afford several other examples of a similar nature.

Diet and Infant Mortality.—We have discussed in Chapter VII the high mortality of the nursing young on low-protein diets. One of the most interesting observations made in connection with our rickets studies was in regard to the condition of nursing females on low-calcium diets. Rats on diets comparable to Lot 2785 presented a highly characteristic picture. Even though the animals did not present an abnormal appearance, which is frequently the case on low-calcium diets, they were, nevertheless, in a state of great nutritional instability. Their vitality was much below that of rats on satisfactory diets; their weakness was shown in a spectacular manner when nursing their young. They were nervous and apprehensive, and in walking extended their legs and held their bodies higher than is the case with normal animals. Their gait was unsteady and slightly tottering. When they were at rest they had difficulty in rising to their feet. This condition did not usually appear while the females were nursing their first litters, but after the second litter had been nursed for about 15 to 16 days, the symptoms usually appeared. The skeletons of these rats were very fragile and could only be removed with difficulty from the cooked flesh without fragmenting. Many of the bones broke into small pieces as soon as they were touched.

The young did not develop in a normal manner on these low-

TABLE XXXIV

SHOWING THE RATE OF GROWTH OF YOUNG RATS WHEN THE DIET OF THE MOTHER IS OF FAIRLY SATISFACTORY TO GOOD QUALITY

No. of Experiment.	*Wt. of Mother After Young Were Born. Grams.*	*Wt. of Mother 10 to 19 Days Later. Grams.*	*Wt. of Mother at End of Nursing.*		*No. of Young.*	*Weight of Young at Different Ages.*					
			Days	*Grams.*		*Days.*	*Grams.*	*Days.*	*Grams.*	*Days.*	*Grams.*
1236.....	142	... ...	40	146	7	16	133	30	286	40	544
2811.....	205	19th day... 225	28	215	10	19	225	28	387	..	...
2069 *	215	10th day... 232	22	205	4	10	100	22	197	..	...
2767.....	270	10th day... 275	29	270	6	19	163	29	330	..	...
2153 *	175	15th day... 182	28	192	5	15	137	28	277	..	...
2436.....	200	... ...	32	185	8	18	188	32	420	..	...

* The diets of lots 2069 and 2153 were less satisfactory than the others. On these two diets the animals in the second and succeeding generations became progressively stunted.

TABLE XXXV

COMPOSITION OF DIETS USED IN EXPERIMENTS DESCRIBED IN TABLE XXXIV

No. of Experiment.	*Beefsteak Grams.*	*Wheat Grams*	*Maize Grams.*	*Rice Grams.*	*Oats Grams.*	*Peas Grams.*	*Navy Beans Grams.*	*Casein Grams.*	*Dextrin Grams.*	*Milk Powder* Grams.*	*Butter Fat Grams.*	*Wheat Flour† Grams.*	*Corn Meal‡ Grams.*	*Cabbage§ Grams.*	$CaCO_3$ *Grams.*	NaCl. *Grams.*	KCl *Grams.*	*Cod Liver Oil Grams.*
1236..	50.0								43.5		3.0				1.5	1.0	1.0	
2811..		25.0	11.5	9.5	9.5	9.5	9.5	10.0		10.0	3.0				1.5	1.0		
2069..	10.0			8.0	8.0	8.0	8.0					30.0	15.5	10.0	1.5	1.0		
2767..		30.0	19.0	9.5	9.5	9.5	9.5	10.0							0.5	1.0		1.0
2153..	10.0			9.5	9.5	9.5	9.5					30.0	19.5		1.5	1.0		
2436..	10.0	30.0	19.0	9.5	8.5	8.5	8.5				3.0				1.5	1.0		

* Merrill-Soule Whole milk powder.
† Bolted wheat flour.
‡ Degerminated cornmeal.
§ Desiccated cabbage, subsequently steamed and dried.

TABLE XXXVI

SHOWING THE STUNTING EFFECT ON THE NURSING YOUNG OF DEFICIENCY OF CALCIUM IN THE DIET OF THE MOTHER.

No. of Experiment.	*Wt. of Mother After Birth of Young. Grams.*	*Wt. of Mother 10 to 20 Days Later.*		*Wt. of Mother at End of Nursing.*		*No. of Young.*	*Weight of Young at Different Ages.*					
		Days.	*Grams.*	*Days.*	*Grams.*		*Days.*	*Grams.*	*Days.*	*Grams.*	*Days.*	*Grams.*
2785 *	165	20	157	35	165	3	20	73	35	162	..	...
2733 †	150	13	150	34	151	4	13	48	34	151	61	184
2069 ‡	215	10	232	22	205	4	10	100	22	197	..	...
2732 §	155	18	160	45	150	6	18	90	34	210	54	247 (Five young)
2767 ‖	270	19	275	29	270	6	19	163	29	330	..	...
2782 ¶.....	182	14	160	30		8	19	109	30	172	39	210
2436 **	200	..	...	32	185	8	18	188	32	420	..	...

* Diet consisted of wheat, maize, rice, oats, peas, beans, casein, NaCl, butter fat and cod liver oil. (See p. 423, etc.)

† 2733 Diet same as 2785 except that cod liver oil was the only fat added, and casein was substituted by beefsteak.

‡ Diet consisted of beefsteak, oats, peas, beans, rice, cabbage, wheat flour, corn meal, $CaCO_3$, and NaCl.

§ Diet similar to 2733 but with less cod liver oil. (See p. 423.)

‖ Diet closely similar to 2785, but contained 0.5 per cent. of added $CaCO_3$. Note benefit to growth of young.

¶ Diet like 2785 except that casein was omitted. (See p. 423.)

** Diet similar in properties to the others used in these experiments but with the optimal amount of calcium carbonate added (1.5 per cent.). Note the remarkable stimulating effect on the growth of the young.

TABLE XXXVII

COMPOSITION OF DIETS USED IN EXPERIMENTS DESCRIBED IN TABLE XXXVI

No. of Experiment.	Beefsteak Grams.	Wheat Grams.	Maize Grams.	Oats Grams.	Peas Grams.	Navy Beans Grams.	Rice Grams.	Casein Grams.	Cabbage* Grams.	Wheat Flour† Grams.	Corn Meal‡ Grams.	Butter Fat Grams.	Cod Liver Oil. Grams.	$CaCO_3$ Grams.	NaCl Grams.	$NaHCO_3$ Grams.
2785		25.0	20.0	10.0	9.5	9.5	10.0	10.0				2.0	1.0		3.0	
2733	10.0	30.0	19.5	8.5	8.5	8.5	9.5						3.0		1.0	1.5
2069	10.0			8.0	8.0	8.0	8.0		10.0	30.0	15.5			1.5	1.0	
2732	10.0	30.0	19.5	9.5	9.5	8.5	9.5						1.0		1.0	1.5
2767		30.0	19.5	9.5	9.5	9.5	9.5	10.0					1.0	0.5	1.0	
2782		30.0	20.0	11.0	11.0	11.0	11.0					2.0	1.0		3.0	
2436	10.0	30.0	19.5	8.5	8.5	8.5	9.5					3.0		1.5	1.0	

*Cabbage desiccated, steamed and subsequently dried.
†Bolted wheat flour.
‡Degerminated corn meal.
§Norwegian cod liver oil employed in all experiments.

TABLE XXXVIII

SHOWING WEIGHT CHANGES OF FEMALE RATS AND THEIR YOUNG DURING THE NURSING PERIODS AS INFLUENCED BY THE CHARACTER OF THEIR DIET

No. of Experiment.	*Weight of Mother After Young Were Born.*	*Weight of Mother 10 to 18 Days Later.*		*Weight of Mother When Through Nursing.*		*Weight of Young at Different Ages.*						*No. of Young.*	*Generation on Diet.*
	Grams.	*Days.*	*Grams.*	*Days.*	*Grams.*	*Days.*	*Grams.*	*Days.*	*Grams.*	*Days.*	*Grams.*		
2436 *.....	200	17	250	28	222	17	140	28	258	..	...	6	1st
"	257	11	277	25	265	11	185	25	200	..	...	4	2nd
"	182	..	...	30	180	16	132	30	305	..	...	6	3d
"	180	17	195	30	195	17	170	30	335	..	...	6	4th
2153 †.....	220	..	...	31	225	11	170	31	400	..	...	10	1st
"	175	15	182	28	192	15	137	23	208	28	277	5	1st
2069 ‡.....	200	27	172	35	177	12	115	27	243	35	342	7	1st
"	167	31	173	38	175	17	135	31	275	38	390	6	2nd
"	135	14	150	28	145	14	90	23	202	..	...	4	3d
2360 §.....	175	31	175	..	...	12	95	19	148	31	265	6	1st
"	142	22	152	33	154	14	120	27	225	33	350	7	2nd
"	115	30	128	37	132	..	...	30	209	37	262	7	3d

* Diet very good. It consisted of wheat, maize, oats, rice, peas, beans, beefsteak, NaCl, $CaCO_3$, and butter fat. Full formula is given on page 425. There was no deterioration observable in the 4th generation.

† The females of the 2nd generation never had any young. The diet consisted of milled wheat flour and maize meal, polished rice, oats, peas, navy beans, beefsteak, NaCl, and $CaCO_3$. The first generation did very well for about eight months, then failed rapidly. The 2nd generation failed very early without reproducing. Formula on page 425.

‡ Similar to 2153, but contained 10 per cent of cabbage. Family at first did well, but deteriorated rapidly with each succeeding generation. The 4th generation were in poor condition and had no young.

§ Similar to 2436, but contained no butter fat, and was alkalinized with $HNaCO_3$. Fertility good and infant mortality low, but each succeeding generation was decidedly smaller than its parents. The fourth generation was discarded in stunted condition at the age of 2½ months. These experiments show the value of continuing observations over several generations, for they illustrate the tendency toward degeneration when the diet is faulty in even a slight degree.

TABLE XXXIX

Composition of Diets Used in Experiments Described in Table XXXVIII

No. of Experiment.	*Beefsteak Grams.*	*Wheat Grams.*	*Maize Grams.*	*Oats Grams.*	*Peas Grams.*	*Navy Beans Grams.*	*Wheat* Flour Grams.*	*Corn† Meal Grams.*	*Rice‡ Grams.*	*Cabbage§ Grams.*	*Butter Fat Grams.*	$CaCO_3$ *Grams.*	$NaHCO_3$ *Grams.*	NaCl *Grams.*
2436....	10.0	30.0	19.5	8.5	8.5	8.5			9.5		3.0	1.5		1.0
2153....	10.0			9.5	9.5	9.5	30.0	15.5	9.5			1.5		1.0
2069....	10.0			8.0	8.0	8.0	30.0	15.5	8.0	10.0		1.5		1.0
2360....	10.0	30.0	19.5	9.5	9.5	9.5			9.5			1.5	1.0	

* Bolted wheat flour.

† Degerminated corn meal.

‡ Polished rice.

§ Desiccated cabbage, subsequently steamed and dried.

calcium diets, even though the mother sacrificed much from her skeleton and tissues. They remained small and many developed the "pot bellies" which are characteristic of poorly nourished little rats. The mothers did not kill the young, which was frequently the case on low-protein diets.

Hartwell (1922) reported data on the effect of diet on mammary secretion. She attributes spasms in the nursing young to an excess of protein in the diet of the lactating mother. Several other investigators have reported this condition in nursing rats, and found it to occur most frequently when the diet is low in vitamin B. She (1924) states that with diets rich in protein, the lactating rat must have a greater supply of vitamin B than when it is on a lower protein diet. Simmonds (1924) has described numerous experiments in which rats were fed as much as 67 per cent. of protein but with the diet chemically complete in other respects, and on which the young were weaned in excellent condition. In no instance was a spasm observed in a young rat nursing a mother whose diet contained an excessive amount of protein but which was otherwise satisfactory.

Composition of Milk of Pellagrous Women.—Voegtlin and Harries (1920) stated that pellagra sometimes occurs in breast-fed infants. They made an investigation of the milk of pellagrous women and reported that "women with well-marked pellagra yield milk which, as far as its composition with respect to the known milk constituents is concerned, does not show a sufficient deviation from normal to account for the disease in nursing infants."

Quality of Breast Milk Dependent on Diet.—During the World War the state of malnutrition among the children of Europe caused great concern. Chemical investigations of the composition of milks produced by women whose babies were not thriving were reported by Momm and Kraemer (1918-19). The fat content was essentially normal. The observation of Kaupe (1918-19) on mothers' milk during the World War is especially interesting. He states: "The absolute amount of breast milk should be sufficient to yield normal increases in weight, but such was not the case. If it cannot be the fat content of the mothers' milk which causes so little increase in the weight of the babies, and if it cannot be shown by us that our mothers give proportionately too little milk, and finally, if it is quite impossible to show that there is a serious lowering of the percentage of carbohydrate of protein, then we must admit that the mothers'

FIG. 33.—This illustration shows the great differences in the size, stage of development and vigor in young rats, which may result from faults in the character of the diets of the mothers which they are nursing. These three rats were the same age. The mother of the two little ones received a diet which was deficient in the quantity and quality of its proteins.

milk is undergoing changes which we know nothing of." The young did not gain in weight normally. The average volume of milk produced by these mothers during the eleventh to the thirteenth day after their infants were born was 440 c.c. Weeks or even months frequently passed before the children regained the weight which they lost immediately following birth. This was true even when there was no evidence of disease. Kaupe was unable to explain the nature of the changes which rendered the breast milk inadequate and attributed to psychic influences the deterioration of its nutritive value. Due to the War, he was not acquainted with the experimental work in America which showed how greatly the diet of the mother may influence the nutritive value of the milk.

Breast Milk as a Food.—Studies of infant mortality show convincingly that breast feeding in early life is a distinct advantage to the child and affords it a better chance of survival through the first year. Woodbury (1926) has analyzed the causes of death among infants and finds an excess mortality among the artificially fed. Death was more frequent from gastric and intestinal diseases, conditions which are especially associated with improper feeding. He also finds the rates from all the other principal causes to be greater among the artificially fed than among breast-fed infants. The death rate from respiratory diseases averages 85 per cent. higher in the artificially fed than among the breast-fed babies, and all other causes rate from 2 to 5 times greater in artificially fed than in the naturally fed.

There can no longer remain any doubt, however, that human milk has been over-rated with respect to its food value. Recent researches show clearly that it is not necessarily a perfect food. The quality and quantity depend, in the human mother as in other species, in great measure on the nutritive condition of the lactating woman. Macy and Outhouse (1928) have made an important contribution to our knowledge in this field. They have shown that breast milk is a food varying greatly in quality, and that it may fall short of meeting the nutritive requirements of an infant. While death may not occur, growth and development may be below the optimum. They have shown from the most critical studies that a reduction in liquid intake, failure to empty the breasts completely at given intervals, and overwork have a tendency to lower quantity production. The milk from the same individual may vary considerably from time to time because of these factors, as well as from faulty diet of the

lactating woman. The consequences of faulty diets have been abundantly demonstrated in the case of lactating experimental animals. Macy and Outhouse found that milk from different women may vary, and that this individuality in composition may persist throughout lactation and still support normal growth in babies. They have made painstaking feeding tests on young rats with human milks from women who were under careful supervision. These breast milks were by no means always adequate in their vitamin content, due to deficient intake in the women's food. Their results confirm those of Andrews, elsewhere referred to, on the deficiency of milk of women with beri-beri.

Sure (1927) has emphasized that the requirements for certain vitamins during pregnancy and lactation, with their drain on the maternal supply, are unexpectedly large,—which emphasizes the importance of a diet of superior quality for pregnant and lactating women. Macy and Outhouse (1928) using milk pooled from 10 to 16 women on average American dietaries, demonstrated that at least for rats human milk does not contain a demonstrable amount of the anti-beri-beri vitamin, whereas cow's milk does contain a small amount. They emphasize that lack of satisfactory food, exercise, fresh air and sunshine, and a condition of constant mental and physical fatigue of the mother during the prenatal and postnatal periods may cause ill effects of various kinds. They emphasize that a resolute attitude toward the nursing of an infant by the mother would greatly reduce infant mortality, and that the mother should avoid overwork, undue anxiety, excitement and irregularity, and that her diet should be most carefully planned to make it complete.

Importance of Protective Foods in Diet.—In a civilized country where food is abundant and people are prosperous, people tend to cater to the sense of taste, and in so doing frequently render their diet inadequate through unwise selection of food; in countries where food is scarce and but few articles are available, they tend to regard food mainly as an agency to relieve suffering through allaying the pangs of hunger. In the latter case the diet is generally faulty because of its simplicity and the absence of an effective supplemental relation among the different articles eaten. The experience of Andrews in the Philippine Islands is a case in point. Hess (1918) observed in the Columbus Hill District of New York that negro women whose diets were derived almost exclusively from milled cereals, tubers and muscle meats, failed to nourish their infants satisfactorily.

The occasional occurrence of scurvy in breast-fed infants represents an extreme example of specific starvation of the mother for a single dietary factor and the resulting injury to her nursing infant. Many women while nursing infants take diets for days or weeks derived in great measure from tea and toast, sugar, and other articles of food equally unfit for the elaboration of milk. The nursing mother should always take a diet which will insure a satisfactory composition of her milk. This can best be realized by including in her menus liberal amounts of milk and of salads (the protective foods). Salads should be composed in great measure of fresh foods such as lettuce, cabbage, celery, tomatoes, etc., and a liberal supply of citrus fruits. A liberal use of leafy vegetables, such as spinach, beet greens, turnip greens, kale, and brussels sprouts, will also tend to insure a calcium supply, as well as aid in preventing constipation. It is not enough for a diet to yield a proper caloric value, furnish protein according to the long accepted standards, and afford variety and palatability. The specific dietary properties of the foods which enter into the diet are of paramount importance and must be given consideration. When the milk supply is limited, which is the case in China and Japan, mothers frequently nurse their children four to five years. They gradually accustom the children to a diet to which it would be impossible to restrict a child at an age at which American children are receiving cow's milk.

This chapter on the pregnant and nursing mother strikes the keynote of this book. It affords proof of the existence of, and points the way to a remedy for one of the greatest sources of human inefficiency and suffering. Since it touches the root of the evil effects of one of the most colossal errors of civilization, this chapter serves to illustrate most vividly the far-reaching importance of scientific nutrition in preventive medicine. It is only within the last few years that the diet of the human nursing mother in its relation to the quality of her milk has received the attention which it deserves, even by investigators. Until the science of nutrition forced upon us the importance of the selection of foods, it was taken for granted that whatever milk was formed by the mammary gland was of good quality. A study of the data makes it apparent how dependent the nursing mother is on the character of her diet as regards the quality of the milk which she will produce. When her diet contains proteins of high biological value, an abundance of the vitamins, inorganic elements in proper amounts, and a source of energy in the form

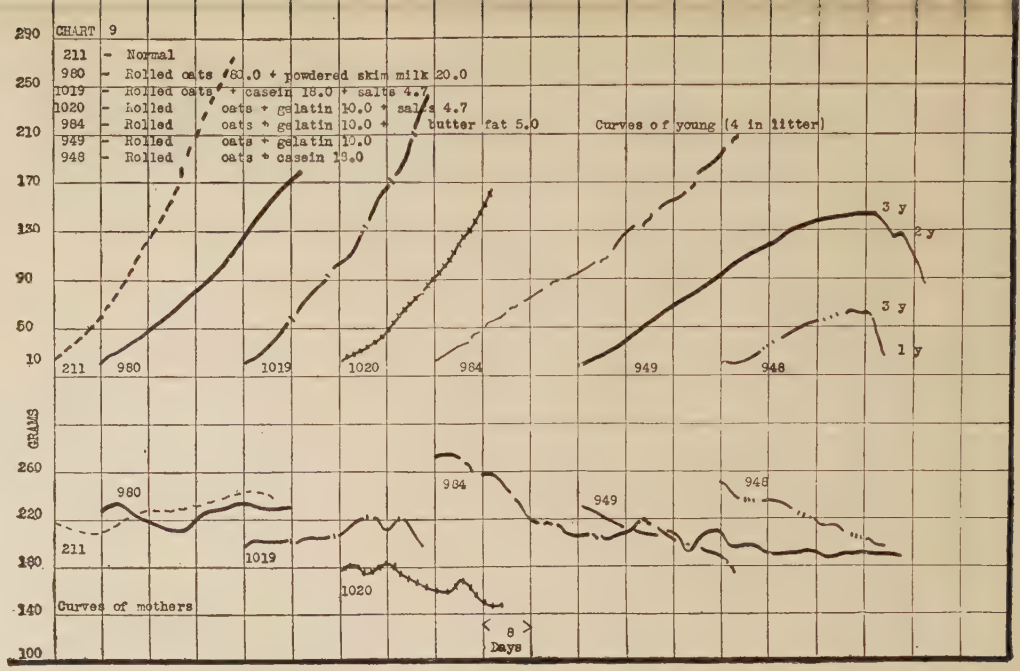


CHART IX.—The curves at the top of this chart show the rate of growth of litters of four young each, when they were nursed by mothers taking the diets listed. No. 211 is an excellent diet containing a liberal amount of dried milk. On this the mother is able to induce rapid growth in her young.

Lots 980, 1019 and 1020 were given rolled oats and a protein supplement, either gelatin or casein, together with a mineral salt addition. Lots 984, 949, and 948 did not receive the supplementary salt additions. The inferiority of their young as contrasted with the first named group is very marked. Lot 984 was given a supplementary supply of vitamin A over that which was contained in the seeds. This prevented the early failure of the young as happened in Lots 948 and 949 which were not given an additional supply of this substance over what was furnished by rolled oats. These curves show clearly that the quality of the mother's milk is profoundly influenced by the character of her diet. If she is selectively fasted for mineral elements, vitamin A, vitamin B, or vitamin C, or if the proteins of her diet are not of good quality her milk is deficient in the substances which are wanting, relatively, in her diet. It is of the utmost importance that the diet of the nursing mother should be of very excellent quality with respect to all its parts.

The curves at the bottom of the chart are also of great interest. Note that those mothers which were fed rolled oats supplemented with a protein and a salt mixture were able to sustain their body weight much better than could those which were fed oats and a protein or oats, protein and butter fat. The draft upon the vitality of the mother while nursing young will be greatly relieved by making her diet highly satisfactory. Note that Lots 948, 949 and 984 lost weight much more rapidly than did those mothers whose diets were better constituted for the promotion of growth in their young.

of carbohydrates and fats, she produces a milk which will induce optimum gains in the weight of her young. In so far as her diet falls short in these essentials will the quality of her milk be reduced. The nursing mother cannot, except in a very limited degree, put into her milk from her bodily reserves that which she does not receive in her food supply.

CHAPTER XXVII

DIETARY HABITS OF MAN IN DIFFERENT PARTS OF THE WORLD

From earlier chapters it will be understood that until the last few years students of nutrition necessarily had an inadequate understanding of the principles which underlie the science. Several writers were inspired with enthusiasm for special types of diets. Each had, therefore, a polyphemic eye for one or another nutrient principle which overshadowed others in importance.

The prevalence of skeletal defects, such as rickets and diseases of the teeth, are now extraordinarily common among certain peoples, but relatively rare among others. There is a widespread belief that these defects are on the increase, and that there is something in the dietary of modern, civilized man, especially in regions where commerce is most developed, which tends to favor physical deterioration. We have on the one hand the evidence gained through experimentation, in which the analogue of diseased conditions in man are produced at will in the animal, to aid in interpreting the problem of the importance of diet in preventing skeletal defects. On the other hand, we may study human experience as far as possible, with a view to discovering the kinds of food which peoples have subsisted upon at various times and in various parts of the world, and correlate with these findings the condition of their bones and teeth. We have brought together in this chapter certain information which has a bearing on this subject.

Primitive man ate everything he could secure which was edible, including the flesh of mammals, fish, birds and shellfish, also eggs and insects, as well as fruits, berries, and fleshy roots, and nuts and a few other seeds of plants, among which were the seeds of those grasses which have since been developed into our cereal crops. Huntington pointed out (1915) that extensive agriculture was impossible until after animals were domesticated. Such seed as was produced was borne on isolated and scattered stems and was difficult to harvest in appreciable quantities. One excep-

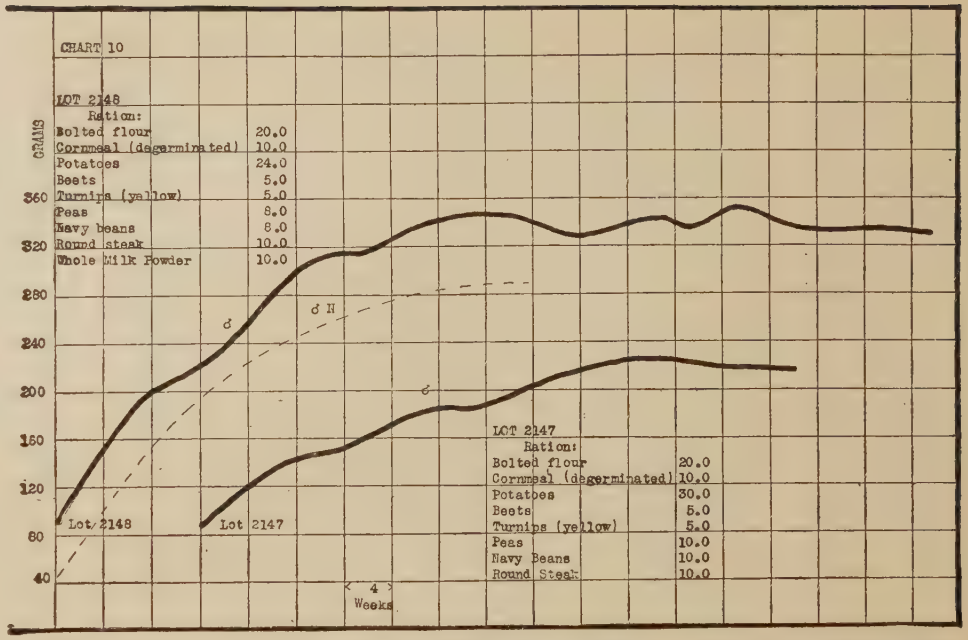


CHART X.—The two curves in this chart serve to illustrate the remarkable superiority of whole milk as a supplement to a mixture of cereal grains, legume seeds, tubers, and fleshy roots as compared with round steak, a muscle meat. Lot 2147 had a complex vegetable diet of the type described, supplemented with a liberal amount (10 per cent) of beefsteak. Their growth was slow and the animals never reached the full adult size. They also developed early in life the appearance of old age. Lot 2148 had essentially the same diet but supplemented with 10 per cent of whole milk powder. This served as a source of mineral salts and of vitamin A, in which diet 2147 was deficient, and made possible much more rapid growth, larger final size and much longer life. These curves illustrate the importance of the proper selection of foods, so as to be highly satisfactory, rather than the adherence to a diet which is somewhat faulty in one or more respects.

tion was the wild rice which grew in the water and was protected to some extent from animals. Rice was harvested, therefore, from very early times, in parts of Asia and in some of the Northern States of America. Grass seeds, such as cereal grains, were sought for at ripening by birds and the harvest time was always short. All the higher apes ate more or less of tender leaves which had mild flavors. After man reached the stage of development when food was regularly cooked, he was able to eat coarse vegetables of the leafy type in greater variety and in larger amounts than when he had to eat them raw, because of the difficulty of digesting some of them. Pot-herbs early became a regular part of the food of human beings as they advanced toward civilization.

Dietary Habits of Early Egyptians.—Ruffer (1921) states that the Egyptians were nicknamed "eaters of bread," and to them, as to the Hebrews, bread was synonymous with food. It was made from spelt (which is a kind of wheat), bearded wheat, or barley. Cereals were cultivated from early times. Flour was prepared in the predynastic period by grinding grain between two stones or pounding it in a mortar. Ruffer comments that the teeth of the soldiers must have been severely tried by their daily ration of four pounds of bread because of its coarse texture. Attrition was intensified by the consumption of raw vegetables. Lentils, beans, artichokes, asparagus, beet root, and cabbages were doubtless cooked, but onions, cucumbers, garlic, radishes, and turnips were eaten raw. The chief fruits of the Egyptians were grapes, figs, dates from the dûm palm, pomegranates, melons, carobs, olives, apricots, and the seeds of marshy plants. With the exception of dates and the seeds of the water plants, the fruit eaten had little or no effect on the teeth, for although the rich people had large fruit gardens, Egypt has never been an extensive fruit-growing country. Egyptians of the poorer class, like the fellah, probably tasted little fruit except dates and melons.

The diet was not wholly vegetarian, for the predynastic refuse heaps of El Toukh have yielded many remnants of animals which served as food. Cattle were kept as far back as Egyptian civilization can be traced, and many wild animals and birds were kept in captivity and artificially fattened. The Nile and its canals were inexhaustible sources of fish. Salted and pickled fish were favorite articles of food. The following inscription of Beha in the eighteenth dynasty refers to the diet of children:

"The children were great and small-sixty . . . they all consumed 120 ephahas of durra, the milk of three cows, fifty-two goats, nine she asses, a hin of balsam, and two jars of oil." According to this, the diet of the well-to-do children consisted in great measure of bread and durra, milk, and oil. The poorer children were given simple cooked foods which could be grilled before the fire, roots of land and marsh plants, sometimes raw and sometimes roasted. The following is a quotation taken from an inscription during the nineteenth dynasty: "The king's messengers had good bread, ox flesh, wine, sweet oil, fat, honey, figs, fish and vegetables every day." The condition of the teeth of the ancient Egyptians on these diets is described in Chapter XXVIII.

Pastoral Nomads.—The Jews, under the leadership of Abraham and of Lot, migrated from the dry plains of Mesopotamia with their flocks and herds and appropriated to their own use the better watered lands of Palestine. They were a good example of a people who subsisted almost solely upon the products of an animal industry. They likewise illustrate an incident which has been many times repeated in human history, viz., a pastoral people who were sufficiently vigorous and aggressive to dispossess their neighbors of their lands. David, the self-reliant and brave youth, the great captain and the national hero, in whom all the noblest elements of the Hebrew genius were combined, was the product of a shepherd tribe.

A scant water supply makes the nomad's subsistence very precarious; it keeps him persistently on low rations, while the drought that burns his pastures and dries up his well brings him face to face with famine. According to Semple (1911) the daily food of the Bedouin is meal, cooked in camel's milk which has been soured, and to which bread and meat are added only when guests arrive. The daily food of the shepherd agriculturist of the Kuen Lun margin of the Takla Makan desert is bread and milk; meat is eaten only three or four times a month. The Tartars eat all flesh of animals dying a natural death; in summer it is sundried for winter use because at that time the Tartars live almost exclusively upon mare's milk, which is then abundant. Jenghis Kahn ruled that neither blood nor entrails nor any other part of the beast which might be eaten should be thrown away. Scarcity of food among the Tibetan and Mongolian nomads is reflected in their habit of removing every particle of meat from the bone when eating. The privations and

hardships of life in the desert and steppes discourage obesity. The Koko-nor Mongols of the Tibetan plateau are of a slight build, and never fat. The Bedouin's physical ideal of a man is one who is spare, sinewy, energetic and vigorous—"lean-sided and thin," as an Arabian poet has expressed it. The Bedouins of Arabia buy flour, clothing, and coffee, and barley for horse feed, and pays for them largely with butter and male colts. Harrison (1924) has written a most interesting account of his experience in Arabia. He describes the Bedouin of the desert as "the lean, indefatigable nomad with the eyes of a falcon, but proud, hungry and indescribably dirty."

Pastoral Arabs.—The diet of the pastoral Arabs consists in great measure of milk supplemented with moderate amounts of meat, cereals and dates. In settled agricultural districts, pomegranates, melons, and a few other things enter regularly into the food supply. The milk used is derived from goats, sheep and camels; it is soured promptly and eaten as curds, because of the impossibility of preserving it otherwise in a warm climate. Cheese and dried curds are also staple articles of diet. This regimen represents a diet largely of a carnivorous nature but supplemented with more or less vegetable foods. It is of the greatest importance to these people that their diet does contain much milk, since it is not possible for them, because of climatic conditions, to profitably grow farm crops. They could not subsist upon a meat, cereal and fruit diet without becoming inferior physically.

According to the Encyclopedia Britannica (vol. 2, p. 285, 11th Ed.), "Physically the Arabs are one of the strongest and noblest races of the world." Baron de Larrey, surgeon-general to Napoleon on his expedition to Egypt and Syria, wrote: "Their physical structure is in all respects more perfect than that of Europeans; their organs of sense are exquisitely acute, their size above the average of men in general, their figure robust and elegant, their color brown; their intelligence proportionate to their physical perfection, and without doubt superior, other things being equal, to that of other nations." In the same article it is further stated of the Arabs that "Simple and abstemious in their habits they often reach an extreme yet healthy old age; nor is it common for them for the faculties of the mind to give way sooner than those of the body."

The use of very liberal amounts of milk is the rule among the people of Northern Africa, Arabia, Mesopotamia, the Balkan

States, and throughout wide areas in Asia. Wherever dairy animals are abundant in proportion to the population and their products form the staple articles of diet, fine physical development is seen without exception.

Douglas (1911) has written an interesting account of the use of fermented milks in the human dietary. The sour milks used so extensively throughout the Far East are variously designated as sheneena, leben, yohourt, kephir, koumiss, matzoon, and dadhi. They are derived from the milk of goats, sheep, cows, and mares. The fermentation is not purely a lactic acid transformation of the milk sugar, for it also includes an alcoholic fermentation. All these sour milks contain a relatively high acidity due to lactic acid, as well as some alcohol.

Tropical Agriculture.—Smith (1919) in discussing the world's food resources points out that the Panama Indians belong to another world, the tropic world. They have not developed from savagery through pastoral or animal-tending states. This is the Aryan method. The tropical people appear never to have used animals to any important extent, but developed a hand agriculture. They depended largely on starchy roots, sweet potato, yam, manioc, taro, caladium, and other roots for food, which have been cultivated so long that they have lost the power to produce seed. The banana, corn and sugar cane have been important additions to this untilled, soil-producing agriculture, which has, with the jungle, alternately occupied parts of tropical America for a length of time that no one knows. The tree crops have also offered great possibilities for food. In fact, tropical agriculture is to a surprising extent an agriculture of tree crops. Trees produce coffee, tea, most of the spices, and cinchona; they also give cocoa, coconuts, palm nuts of several varieties, and Brazil nuts. Coconuts and Brazil nuts as sources of fats are becoming increasingly important. From the sago palm the natives make sago, a staple starch, and from the sugar palm in some parts of the tropics they also make sugar. The orange and avocado are also important fruits.

Indian Dietaries.—McCay (1912) reported an elaborate study of the diets of the peoples of various parts of India. He concluded that a liberal supply of protein was the factor of greatest importance in determining the success of the human dietary. It is now apparent that the protein moiety is but one of the several indispensable components of an adequate food supply, all of which are indispensable and must be viewed as of approximately

equal importance. McCay's observations leave no room for doubt that the pastoral peoples of India are vastly superior in strength, health and manly qualities generally, to other classes of Indians. In those parts of India where the diet is largely derived from cereals, the physical development of the people appears to be the poorest; the rice eaters of Bengal illustrate this well. The details regarding the components of the diets of the different classes studied by McCay are not sufficiently complete to serve our present purpose, since he was interested primarily in the amount of protein which the different diets supplied. When his investigations were made it was not deemed of importance to list the individual food-stuffs eaten and their quantitative relations. *This is regarded to-day as the most essential thing to know when one is called upon to evaluate a dietary.* McCay's studies, however, based upon personal observations, and on the reports of officers of the British Army to their Government concerning the fitness of the men in different parts of India for military service, forms one of the strongest confirmations of the applicability to human experience of the principles of nutrition discussed in this book.

The most important milk product in India is ghee, or clarified butter obtained from the milk of buffaloes. It is sterilized, so is more suitable than butter for the hot climate. It is usually so adulterated with fats and oils that Europeans prefer butter. India imports some butter, and condensed and preserved milk. Many Indian women purchase condensed milks for their infants and children. In Bengal, nearly two million people are supported by catching or selling fish. It is said that 90 per cent. of the population of western India is dependent upon fish for sustenance.

The banana or plantain is cultivated throughout India, except in the extreme northwest. The name banana is seldom used in India except by a few English people. All kinds of this fruit are indiscriminately called plantains. After mangoes, plantains are the commonest and most highly prized of Indian fruits. Bhattacharyya (1928) has written an interesting account of Indian dietaries.

Dietary Habits of Polar Eskimos.—One of the most interesting groups of people in the world from the dietary standpoint are the Polar Eskimos. Their diet appears to be extremely poor in calcium and excessively rich in phosphorus, a relationship which works to the detriment of the skeletal system under ordi-

nary dietary practices in temperate regions. They live on the northwest coast of Greenland, contiguous to the waters of Smith Sound and are the northernmost people in the world. Their food, fuel and clothing come almost solely from animals; only rarely do they eat their native plants. Every animal supplies a part of the diet, and no part of the carcass is discarded. The land animals, although furnishing much of the clothing, constitute a relatively small part of the Eskimo food supply. The ptarmigan is the only land bird which plays any part in the dietary. It is upon the sea birds and sea animals that they depend for clothing, and for practically all their food and fuel (Ekblaw, 1921).

Ekblaw, who lived among the Polar Eskimos, states that fish are an insignificant part of their diet, since they catch salmon only a short time in the year, and no other fish are available. Caribou and musk oxen are not found about the Eskimo villages. Only at intervals of months or even years do the younger people of the tribe have an opportunity to eat marrow of the bones of the land animals. They do not eat the marrow of animals from the sea. The Eskimos do not eat the liver of the polar bear because it is believed to be poisonous, but they consume the livers of other animals with great relish. They are exceedingly fond of the thick, heavy skin of the narwahl and beluga, and seem to need both of these in their food. The millions of dovekies and thousands of murres, eiders and guillemots which frequent the coasts, supply an easy store of meat and eggs which can be readily put away for winter use. The fact that the Eskimos of this polar tribe have such excellent physique, hair, and teeth, and such superb health, without any trace of scurvy, rickets, or other evidences of malnutrition, is interesting in the light of their restricted and simple diet. Hrdlička (personal communication, 1924) has a large collection of skulls and bones from the Eskimos of northwestern Greenland. He states: "Their bones are remarkable for their good development and solidity; while the teeth of the Eskimo as a whole, including those of northwestern Greenland, are probably the best of any large group of people living to-day."

Diet of People of Faroe Islands.—Panum (1847) reported an interesting account of the diet of the people of the Faroe Islands. The only bread which the peasants ate was made of coarse barley which was made into long cakes or rolls without yeast. This bread, with sour milk, and mutton dried in the wind con-

stituted the breakfasts. The mutton was allowed to hang throughout the winter and by spring was regarded as fit to eat. Any unused portion of the carcass became filled with maggots in the summer, but this was regarded as improving the gamy flavor of the meat. This half-rotten meat (flesh, fish or fowl) was called "rast." Large quantities of salted blubber of the Ca'ing whale were likewise eaten, as were cod's heads which also formed a daily article of diet. The peeled stalks of Angelica archangelica were eaten as we would eat strawberries. Panum saw men seventy years of age who had never lost a tooth, but in these cases the crowns of the teeth were worn quite flat as in many of the old British skulls.

Food of Lapps.—The Lapps are scattered throughout northern Scandinavia and the Murman coast. They are an excellent example of the success of a diet largely carnivorous. They eat not only meat, fish and fowl, and eggs of wild birds in summer, but secure a fairly large amount of milk from their reindeer. They are a short and stocky people with good physical development and great endurance. Like the Eskimos, they must exert themselves greatly at times, in order to maintain themselves in their inhospitable land.

Nutrition of People of Iceland.—A most interesting story is the experience of the people of Iceland. This island was settled in the ninth century by colonists from Ireland and Scandinavia who took with them cattle, sheep, and horses. Their diet was practically carnivorous in nature for several hundred years. Martin Behaim (quoted by Burton, p. 32), writing of Iceland about 1500 A.D., stated: "In Iceland are found men eighty years old who have never tasted bread. In this country no corn grows, and in lieu of bread fish is eaten." Burton (1875, p. 154), quoting Pierce, states that rickets, and caries of the teeth, were almost unknown in Iceland in earlier times. Kerguelen (cited by Burton) has left an excellent description of the Iceland trade in 1767. The only money was fish and butter, while an ell of pig-tail (tobacco) equalled one fish. The fisheries were very extensive. Exports were salt meat, beef, mutton, tallow, butter, wool, skins of sheep, foxes and seals, feathers, especially eider down, oil of whales, sharks and seals, fine and coarse jackets of Wadmal, woolen stockings and mitts, stock-fish, and sulphur. The imports were fishing tackle, horse shoes, carpenter's woods, coffee, sugar, tobacco, snuff, beer, brandy, wine, dry goods, flour (wheat and rye) bread, and biscuit.

As an index to the profound changes in the dietary habits of the Icelanders during the nineteenth century we may quote Burton, who visited the island in 1873, and gave an account of the imports at that time: "The cereals, whose consumption ranges from 24-30 bushels a head, are wheat and rye, in grain, flour, and biscuit." "Besides cereals, the stores supply sugars, brown and white candy, hams (rare and no potted meats), sausages and sardines, butter, figs, raisins, prunes and olive oil." The health conditions were good and dental caries were unknown until after about 1850. Stefànsson exhumed 96 skulls from a cemetery dating from the ninth to the thirteenth centuries and presented them to Harvard University. These have been described by Hooton (1918) who found no evidences of caries in any of them. There were but three or four defective teeth in the entire series, and these had suffered mechanical injury. During the last half century caries has steadily increased in Iceland. This corresponds to the importation of grains, and consequent changes in the character of the diet. It will be recalled that the grain eaters of ancient Egypt had very defective teeth.

Diet of Rural Finland.—Sundström (1909) made a study in 1906-07 of the dietaries of the families of 103 farmers and tenant farmers in various parts of Finland. One family, possessed of small land holdings, had nothing but bread, fish and sour milk during the seven days covered by the record. This family was typical of a group in which remarkable uniformity and monotony of the food supply prevailed.

A second type of household, representative of a class, had a diet somewhat better. The following menus were served:

Sunday.	B. Bacon with potatoes, bread, butter, milk. D. Meat, boiled rice, bread, butter, milk. S. Bacon, bread, butter, sour milk.
Monday.	B. Bacon, potatoes, bread, butter, milk. D. Bacon, bread, sour milk. S. Bacon, rye meal porridge, bread, milk.
Tuesday.	B. Bacon, porridge, bread, butter. D. Bacon, bread, butter, milk. S. Bacon, barley porridge, bread.
Wednesday.	B. Bacon, barley grits soup, bread, butter. D. Bacon, bread, sour milk. S. Oatmeal porridge, bread, butter, milk.
Thursday.	B. Bacon, potatoes, bread, butter. D. Pea soup, meat, bread, butter, milk. S. Pea soup, meat, bread, milk.
Friday.	B. Bacon, potatoes, bread, milk. D. Bacon, bread, sour milk. S. Bacon, grits-milk soup, bread, milk.
Saturday.	B. Bacon, potatoes, bread, milk. D. Bacon, potato soup, bread, milk. S. Rice porridge, bread, butter, milk.

A third dietary which shows a somewhat greater variety was that of a Swedish family in Finland. During a seven day period this family had:

Bread—13 times.	Potatoes—8 times.	Curds—4 times.
Milk—10 times.	Grits-milk soup—4 times.	Anchovies—4 times.
Porridge—9 times.	Meat—2 times.	Blood omelet—once.

No other food was served except milk sauce for the anchovies.

Another type of diet used by certain people in Finland is illustrated by a family who had potatoes every meal in the week (21 times), salt fish 9 times, fresh fish 4 times, porridge 4 times, bread 19 times, butter 8 times, milk 17 times, bacon 3 times, pancakes once, sausage once. It is significant that in the Olympic games of 1924 the Finnish contestants carried away a disproportionately large number of honors for the population of their country.

Dietary Habits of People of the Hebrides.—A diet similar to the one used in Iceland has long been customary in certain islands in the Hebrides. The only successful crops are turnips and potatoes. There are one or two cows to each family. Some oatmeal is available, and forms a constant though not an abundant constituent of the diet. Many of the men are engaged in fishing, and the unsalable parts of the cod form a regular and prominent part of the food supply. Milk, cod's head stuffed with cod's livers, turnips, potatoes and oatmeal constitute almost the sole food of the poor crofters. It has already been mentioned that rickets is rare among these people. McDonald (personal communication) states that the teeth of the islanders still adhering to the older dietary practices are far superior to those of Scotch people in the same climate but with the typical modern food supply.

Diet of Carnivorous Man and Animals.—Common observation shows that there are many animals subsisting entirely upon other animals. The wolf, lion and tiger among mammals, and the eagle, owl and vulture among birds, are examples. Nowhere do we find more remarkable physical development than among this class of animals. It is obvious, therefore, that the tissues of one animal contain everything which is essential for the nutrition of another. Those who accepted the old ideas regarding the necessary components of an adequate diet saw no reason to distinguish between different types of carnivorous diets. Muscle meats have dietary properties very different from those of the glandular tissues. Muscle meats are similar in their dietary properties to the

cereal grains, and in order to make them complete foods it is necessary to supplement them with respect to calcium, sodium, chlorine, and all vitamins except G.

The flesh eating of the carnivorous animals is very unlike that of civilized people in the temperate zone. The latter are very fond of the muscle cuts, viz., steaks, chops and roasts, whereas carnivorous men and animals eat blood, glandular structures, bone marrow, fat, and an appreciable amount of bone substance in preference to muscle tissue.

People's tastes vary greatly in the flavors of meats. At one extreme is the practice in the tropics of selling flesh within an hour or so after the animal is slaughtered. The meat is cooked when perfectly fresh. At the other extreme is the practice of hanging meat until it is putrid. Panum (1847) describes this in the Faroe Islands. Zenzinov (1924) describes the custom among the Tungas of northern Siberia of burying geese until they are putrid, when they are eaten and also fed to dogs. In the United States and in parts of Europe people prefer the flavor of meats which have been kept in cold storage for a considerable period. They undergo retrogressive changes and are extensively acted upon by psychrophilic bacteria. The rank flavor of gamy meats and that of fresh flesh are not relished by many people in the temperate zones.

The pronounced deficiencies of muscle tissue as a food-stuff raises the question of the reason for the successful nutrition of strictly carnivorous animals. The explanation is found in the selection which these animals make of the parts of their prey. The custom of the carnivora is to open the jugular veins of a freshly killed animal and suck the blood. This is what one sees when a rat or weasel raids a chicken coop. In temperate and tropical regions, the glandular organs are next selected after blood. In the Arctic region, the second choice, or even the first, may be fat, depending on the previous state of nutrition of the animal,—if it has recently ingested liberal amounts of protein, it may take only fat. This is said to be the practice of polar bears; seals are found stripped of their fat but otherwise are untouched. The demand for energy is so great in these bears that it is necessary to consume fat frequently and in large amounts. Since the protein metabolism is of smaller magnitude, meat of either the glandular or the muscle type may be taken at longer intervals.

Strictly Carnivorous Diets Adequate for Rats.—Blood,

muscle or glandular organs taken alone will not support growth in a rat. A mixture of equal parts of blood and muscle fed to young rats enabled them to make a fair amount of growth, but they remained stunted and failed early. (McCollum, Simmonds and Parsons, 1921). Appropriate mixtures of blood, muscle and bone together with liver or kidney make a fairly satisfactory diet (McCollum and Simmonds, unpublished data). The controlled experiment of the laboratory revealed the secret of success in the nutrition of a class of animals whose excellent physical development on a diet derived solely from animal tissues has often been interpreted as signifying that meat (muscle tissue) is a complete food, and one of exceptional value for promoting vigor. In reality, the muscle cut of meat as the sole article of diet is the poorest part of the carcass. It is due to successful combinations which carnivorous man and animals make which renders possible their nutritional success.

Successful Rearing of Young Lions.—The importance of a proper choice of animal parts in order to make a complete diet is illustrated by the rearing of young lions in captivity. With few exceptions in the past such attempts have proven failures, since the animals developed faulty bones. If they survived, they were deformed and unfit for exhibition. A young lion cannot grow normally when fed upon a piece of muscle meat with a large hard bone attached. When cubs are fed upon liver, fat and bone of a character such that they can eat a considerable amount, and when their diet is varied frequently with small animals which can be consumed entirely, such as rabbits or pigeons, they grow to be strong and beautiful animals.

Scully describes the feeding habits of the lion in the jungle. The zebra is the lion's favorite food. He opens the carcass at the flank, where the skin is thinnest, eats the layers of skin and flesh covering the paunch, and consumes the soft ends of the ribs and the cartilages of the thorax. Then he removes the viscera without breaking the paunch. After eating the liver, kidneys, heart and lungs, together with any fat distributed among the viscera, he carries the paunch and entrails away and hides them. Then he returns to tear from the thigh large pieces of meat which he swallows whole, skin and all. It is well known that the jackal feeds on the animal carcass which the lion has left. The jackal supplements this muscle tissue with eggs, and newly hatched chicks of the ostrich. This practice is so common that the ostrich and jackal are constantly associated in desert

life. Since eggs contain all the vitamins except C, they supplement the raw muscle meats which the jackal obtains, and together with bone, complete his diet.

Toxic Products Produced from Protein.—Any strictly carnivorous diet must consist largely of protein and fat. When derived from the tissues of animals, the carbohydrate moiety is so small and the protein content so high that the bacteria which flourish in the lower intestine will always be of the putrefactive type. The view of Metchnikoff, that the body is injured by absorption of toxic substances formed in the intestine through the agency of putrefactive bacteria, rests upon evidence which can hardly be doubted. The beneficial effects resulting from taking a liberal milk diet, especially milk which has been soured by B. acidophilus, are attested by many people. A striking illustration of the manner in which a diet consisting largely of animal tissues modifies the bacterial flora of the intestine and influences the physical and psychical condition of the animals is given by Herter and Kendall (1909-10). They restricted monkeys to a diet of eggs, and cats to a diet of meat for one or two weeks, then changed them to a diet of milk and glucose. Previous studies had shown that eggs or meat encourage the growth of putrefactive organisms in the colon, whereas milk and glucose stimulate the growth of fermentative and lactic-acid-forming organisms. This is also true of a diet consisting largely of carbohydrates. After one or two weeks on the milk and glucose diet, the animals were returned to eggs and to meat respectively. These intervals were found sufficiently long to change entirely the character of the bacterial flora of the intestine. As the proteolytic or putrefactive types of bacteria began to predominate, which occurred promptly after restricting the monkeys to eggs, the animals became sleepy and rested their heads upon their hands in a bowed position. They were stupid and responded slowly to external stimuli. They took their food very deliberately and manifested little interest in their surroundings. Not infrequently after a hearty meal they would spend much time trying to bite the woodwork of the cage. The urine was of small volume and amounted to approximately half that produced from the milk-carbohydrate diet. As the protein-digesting bacteria became established in the intestine, the amount of products of putrefaction in the urine increased markedly.

When the animals were changed to the milk-glucose diet, both the psychical and physical attitudes underwent a great change.

They no longer held their heads in their hands but assumed an erect posture. They were alert and bright, and showed a keen interest in their surroundings. Their appetites became sharp, and food was consumed with more avidity. The eyes, which were dull and lusterless while on the egg diet, became bright. They no longer chewed the woodwork. The products of putrefaction almost disappeared from the urine. Evidence from human experience seems sufficient to warrant the conclusion that similar changes in the diet of man induce a similar modification in the flora of the intestinal tract. One may safely conclude that excessive consumption of meat with but small amounts of carbohydrates tends to promote the generation of toxic substances which induce lethargy in both man and animals.

Carnivora and Physical Activity.—It appears that the carnivorous peoples resemble the carnivorous animals in their attitude toward physical exertion. When well fed, they are lethargic and remain idle, or do work that requires little exertion; they bestir themselves only when pressed by hunger. Dr. Grenfel states that the Eskimo is an inferior fisherman and will catch one fish to a white man's ten. This we may attribute, in part at least, to intoxication from poisons of bacterial origin due to putrefaction of protein in the intestine.

Dietary Habits of American Indians.—American Indians who inhabited the plains east of the Rocky Mountains and subsisted mainly upon the bison were essentially carnivorous in their habits during the greater part of the year. Catlin (1832) visited a Mandan tribe of about 2,000 living in two permanent villages on the upper Missouri River. They had a fixed abode because they were too few to contend with the plains Indians, and accordingly practiced some agriculture. Nevertheless, he states that "The Buffalo meat, however, is the great staple and 'staff of life' in this country, and seldom, if ever, fails to afford them an abundant and wholesome means of subsistence. There are, from a fair computation, something like two hundred and fifty thousand Indians in these Western regions who live almost exclusively on the flesh of these animals, through every part of the year."

This tribe cultivated corn, pumpkins and squashes. Wild fruits and berries of different kinds were dried and laid away for winter. An important article was the "pomme blanche," a kind of wild turnip which was eaten raw and was much sought after by the migratory Indians. It is stated that the route taken in fol-

lowing the buffalo in their tribal hunts was often determined by the localities where this turnip was most abundant.

The numerically strong tribes migrated in order to keep in contact with the buffalo herds, and did not cultivate the soil. Accordingly, they had vegetable food only as they gathered wild plants on the prairies, and fruits such as plums, choke cherries, wild grape, elderberries, wild raspberries, strawberries, etc., as well as the yellow lotus which grew in abundance in a few sheltered shallow bodies of water. Indian potato and falcata beans were also eaten. The latter produced two different fruits: pods containing small beans to which little attention was paid, and large subterranean beans which were eagerly sought as an article of food on account of their agreeable taste. These beans were collected by meadow mice into hoards of a pint or more in a place, which the Sioux tribe would seek out and take a part of, replacing the amount they took with corn.

The vegetable food of the hunting Indians formed an uncertain and variable proportion of their total food supply. They were essentially carnivorous. Reliable observers such as Lewis and Clark, Parkman and others, testify that in many instances they were fine examples of physical development. Like the Eskimos, they ate the entire edible portions of the animals which served them as food.

Fletcher and La Flesche (27th Annual Report, Amer. Bur. of Ethnol.) described the habits of the Omaha Indians. During the summer and fall months they used the meat fresh and cooked in a variety of ways. During other parts of the year they ate principally pemican. This was made of buffalo meat cut in thin strips and dried very hard, and afterwards pounded in a large wooden mortar until it was made nearly as fine as sawdust, then packed in this dry state in bladders or sacks of skin. Marrow fat was collected from the buffalo bones and put into buffalo bladders for preservation. After it cooled it was very much like tallow but had nearly the flavor of the richest yellow butter. At feasts chunks of the marrow fat were cut off and eaten with the pemican.

Some Indians of the Southwest were more settled agriculturists. They engaged in hunting, but supplemented their animal food with more corn and beans, and ate more wild fruits and wild plants.

Reference has already been made to the perfection of the teeth of persons of advanced years among the plains Indians. Mr.

V. W. McMullen informs us (personal communication) that in a series of about two hundred skulls excavated from the ruins of the old cliff dwellings, which represent the skulls of the ancestors of the Hopi, no carious teeth were found. It is evident that the development of the bones and teeth among these primitive people were far superior to those of the white people living in the same regions to-day. Hrdlička (1908), writing of the Indians of the Southwestern United States and Northern Mexico, stated, "Dental caries is not rare, though much less frequent than among the whites. It is usually a molar or premolar that is affected." There is much reason to believe that since these Indians have become wards of the Government that dietary changes have had much to do with the deterioration of their teeth.

Health of Non-Citizen Indian.—There is no better illustration of the soundness of the views regarding the types of diet which succeed in inducing good nutrition than the experience of the non-citizen Indian of the United States. All who observed the Indians in their primitive state agree that most of them were exceptional specimens of physical development. With few exceptions, however, during two generations they have deteriorated physically. The reason for this is apparently brought to light by a consideration of the kind of food to which they have restricted themselves since they have lived on reservations.

There is no group of people with a higher incidence of tuberculosis than the non-citizen Indians. As wards of the Government, they have been provided with money and land but have in general shown little interest in agriculture. They have lived in idleness, and have derived their food supplies from the agency stores. In addition to muscle cuts of meat they have, therefore, taken large amounts of milled cereal products, cyrup, molasses, sugar, and canned foods such as peas, corn, and tomatoes. In other words, they have come to subsist essentially upon a milled cereal, sugar, tuber, and meat diet. On such a regimen their teeth have rapidly become inferior, and are badly decayed. They suffer much from rheumatism and other troubles which result from focal infections. Faulty dietary habits are, in great measure, to be incriminated for their susceptibility to tuberculosis.

Other classes of Indians who have become successful farmers have not deteriorated as a result of contact with civilization, except in so far as they have suffered from alcohol and venereal infections. The non-citizen Indian has suffered, not because of

contact with civilization, but because he has been forced into dietary habits which are faulty.

Diet of Sioux Indian.—Stene and Roberts (1928) recently made a nutritional study among a typical reservation group of Sioux Indians in South Dakota. These Indians are by preference essentially carnivorous, and even to-day fruits and vegetables form an uncertain and variable part of their food intake. Butter and eggs were not a part of the diet of the primitive Indian, and at present these foods are not much used. Milk also has a minor place in the dietary which abounds in "grease bread" and coffee when the meat ration becomes scanty. Casual observation shows a great prevalence of tooth decay, bow legs, sore eyes and blindness among these families. The observers remark that although no definite conclusions can be drawn as to cause and effect relationship between the diets and the physical deterioration, it is obvious that the dietary deficiencies are of a nature that would be expected to produce the types of physical deterioration found. Lack of calcium, which is essential for skeletal development, shows its effects in the prominence of poor teeth and bow legs. It was not determined whether the sore eyes and blindness were due to trachoma or venereal disease, or might be attributed to ophthalmia because of lack of vitamin A. In view of the alleged relation of vitamin A to the prevention of tuberculosis and of respiratory infections in general, the fact that 85 per cent. of the deaths from known causes were due to these infections suggests that the diet, which is especially low in A, may be a determining factor in these conditions. One-third of the child mortality in the Sioux group studied occurred in a few families whose diet was principally bread and coffee. The fact that these observations were made by trained observers gives them particular value. Stene and Roberts venture the conclusion that both unsatisfactory sanitation and inadequate diets were doubtless responsible for the physical deterioration of the Indians on this reservation. They emphasize that the deficiencies of the diets are of such type and degree that they alone would have been sufficient to account for the physical deterioration.

Diet of Indian School Children.—Former Secretary of the Interior Work (1928) states that the oustanding deficiency in the Indian schools is in the diet furnished the reservation Indian children. The diet is deficient in quantity, quality and variety. The effort has been made to feed the children on a per capita of eleven cents per day, plus what can be produced on the school farm,

including the dairy. At a few—very few—schools, the farm and the dairy are sufficiently productive to be a highly important factor in raising the standard of the diet, but even at the best schools these sources do not fully meet the requirements for the health and development of the children. At the worst schools, the situation is serious in the extreme. The major diseases of the Indians are tuberculosis and trachoma. Tuberculosis unquestionably can best be combated by a preventive, curative diet and proper living conditions, and a considerable amount of evidence suggests that the same may prove true of trachoma. The great protective foods are milk, fruits, and vegetables, particularly fresh green vegetables. The diet of the Indian children in boarding schools is generally notably lacking in these preventive foods. Although the Indian Service has established a quart of milk per day per pupil as the standard, it has been able to achieve this standard in very few schools. At the special school for children suffering from trachoma, now in operation at Fort Defiance, Arizona, milk is not part of the normal diet; the small amount of milk produced is mainly consumed in the hospital where acutely ill children are sent. It may be seriously questioned whether the Indian Service could do very much better than it does without more adequate appropriations.

It is not essential that we retrogress to a state of social, educational or ethical inferiority in order to enjoy the physical fitness frequently seen in half-savage peoples. Too much emphasis has been placed upon the beneficial effects from the standpoint of health, of hardship and exposure to which primitive man was subjected. Hardship and exposure never did any one any good. Freedom from excessive exertion and protection from the elements are both conducive to health. There is no question but that one of the main factors which has tended to reduce civilized man to a state of physical inferiority as compared with his barbarous ancestors are changes in the character of his diet.

Successful Nutrition Among Leaf-eating Animals.—One of the greatest surprises in the history of nutritional research was the discovery by McCollum, Simmonds and Pitz of the unique dietary properties of the leaf as compared with those of the other parts of the plant. The knowledge gained by 1916 had shown that the cereals, tubers, roots, and legume seeds collectively, in any proportions, could not form a satisfactory food supply for any kind of animal with which experiments had been made. The thought naturally suggested itself that in the dietary of peoples

who are largely vegetarian there must be something which supplemented the deficiencies of the seeds, tubers, and roots. It was found that meats could not serve this purpose, since they do not greatly enhance these storage products of the vegetable world with respect to several factors in which they are deficient. Later studies have clearly shown that the leafy vegetables play a very important rôle in the dietary of many Oriental peoples.

It is well known that grazing animals may live throughout their lives on grass or other palatable forage plants. The horse, cow, sheep, goat, and deer, and other grazers, in their early history had no grain, but subsisted entirely upon grass and tender leaves. The bison never had anything throughout life but the buffalo grass.

The Chinese Diet.—Wang (1920) gives an interesting discussion of the Chinese diet. It consists of eggs, meat, fish, fruit, cereals, and a great variety of vegetables. Rice takes the place of bread. Pork is used by practically all classes in all parts of China; fresh pork is such a common food that wealthy people do not eat it. Lamb may be substituted for pork during festivals, but beef is considered more or less sacred and is seldom used for food. The quantity of meat eaten is small; it is cut into small pieces and mixed with vegetables in a variety of ways. Fish and shell fish, including crabs, shrimps, lobsters and oysters, are used extensively.

Unlike Americans, who consider the muscle of an animal almost the only edible part, the Chinese people eat practically every part except the hair. They eat brain, spinal cord, the various organs, blood, and even the skin. Blood is coagulated after it is drawn from the animal and can be purchased in the market in brownish cake-like pieces which look much like liver. Blood is an inexpensive food and is used liberally. Many chickens and ducks are also used. One can purchase in the markets the parts of the fowl which one desires, such as ducks' tongues, or a dozen ducks' heads. Fowl are more expensive than either pork or fish and are, therefore, seldom used by the poor. They are eaten so extensively by the wealthy that they probably furnish the chief source of protein. Geese, pigeons, turkeys, pheasants, and many other domestic and wild birds are also used, but are scarce and are considered delicacies.

The eggs of ducks and pigeons, as well as hens, are used freely in the Chinese diet. They are fried and scrambled, and in addition are used for seasoning, garnishing, making noodles, and pre-

serving. Eggs in one form or another enter into most Chinese dishes. A distinctly Chinese use of eggs is for preservation. At least three different kinds are produced, viz., hulidan, or salted ducks' eggs, dsaudan or fermented eggs, and pidan, the so-called "Chinese old eggs."

It is partly this extensive use of eggs by the Chinese people which makes their diet adequate. They use little or no milk. Cows are employed only for draft purposes. Babies, invalids and the aged are sometimes given human milk. Mothers nurse their children for long periods, and then secure a wet nurse to prolong the nursing until the child is a year or two old. Vegetables are used much more frequently by the Chinese people than by Americans. In addition to spinach, cabbage, potatoes and radishes, many plants and weeds are eaten which are usually not considered food in America. Radish leaves, shepherd's purse, bamboo sprouts and a large number of sea weeds are eaten. The Chinese have developed great skill in cultivating vegetables. There are at least six or seven varieties of spinach, differing from each other in the size of the plants, the size of the leaves, length of the stem, and thickness of the leaf. One which grows most abundantly in central eastern China looks somewhat like cabbage.

There are no less than twenty varieties of soy bean, and thirty or more bean products are manufactured. Adolph (1922) has discussed in detail the many uses which the Chinese make of the soy bean. They regard it as a substitute for meat. He reported (1925) a study of North China dietaries. The data cover the diets of 304 adults and 114 children below 12 years of age, corresponding to a total of 19,777 adult unit days. A large proportion of the individuals studied were engaged in farming. In the following table the catty is equivalent to 1.33 lbs.

In the South and central parts of China where rice is produced abundantly, it takes the place of wheat, but in the north in Pekin and Shantung, wheat, corn and millet seed are used rather than rice. Rice is usually unpolished, since polished rice is an expensive luxury. Ground or whole millet seed is made into cakes or a thin mush and is eaten especially by children. In very poor families instead of meat and other expensive foods, the ration consists chiefly of unpolished rice, or in the north, millet seed or corn, bean curd, green onions, sometimes salt fish, and invariably a large quantity of leafy vegetables, including bean sprouts. The eggs, bean sprouts, blood, and the liberal quantities

TABLE XL

Food-stuff	*Weight in Catties*	*Food-stuff*	*Weight in Catties*
Wheat and wheat products	10,929	Corn	278
Melons (13 varieties)	7,270	Oils	246
Rice	6,207	Fish	188
Green vegetables (33 varieties)	2,207	Turnips, taro, etc.	156
Cabbage	963	Fruit (12 varieties)	155
Leeks	488	Shrimps and shell-fish	142
Onions	279	Sugar and starch	92
Garlic	116	Sweet potatoes	50
Celery	79	Beef, mutton, mule meat	44
Millet	1,877	Chestnuts, peanuts	36
Legumes (soy bean, etc.)	1,520	Milk (goats and cows)	33
Bean curd and other bean products	1,061	White potatoes	29
Kaoliang (Sorghum vulgare)	1,000	Chicken	28
Food accessories (salt, vinegar)	744	Other foods	15
Pork	319	Total	34,913
Eggs	287		

of leafy vegetables, Dr. Wang concludes, have served as the "protective foods" in China.

Wu and Wu (1928) have reported studies on the dietaries of middle class families in Peking. Their data show that the diet of the average Chinese, while adequate in fuel value, is suboptimum in protein and vitamins A and D, but probably adequate in B and C. It is low in calcium and phosphorus. They emphasize that there are probably many cases of mild forms of deficiency which are not recognized, and point out that the difference between the diets of Chinese and those of the Occidentals is able to account for the difference in physique and energy between them.

Food of the Japanese.—Morimoto (1918) states that although rice is the important grain in Japan, the general belief that it is the principal food is true only of the urban population. The rural population use whole barley as their principal food. The soy bean is the most important legume. Buddhists, forbidden to

eat meat of any kind, early discovered that this legume would take its place. The consumption per capita per annum is 0.386 bushels, compared with 5.32 bushels of rice. The daily consumption of cereals is, according to Morimoto, rice 0.117 gallons, barley 0.019 gallons, and other grains 0.010 gallons. The most important vegetables in Japan are sweet potatoes and radishes (daikon). Eighty and eight-tenths kilograms of sweet potatoes and 55.9 kgm. of radishes are used per person per year. The radishes are commonly used as pickles, which are eaten with plain boiled rice.

Plums, mandarin oranges, persimmons and pears are the most important fruits in Japan. Animal protein is mainly derived from fish. Meat as a whole is not widely used. Almost two million people are engaged in fishing and more than 400 kinds of fish are caught in Japanese waters. The average amount of fish eaten per person per year is 17.625 kgm. or 49 gm. per day. Sea foods include both animals and plants; the former includes cuttle fish, octopus and shrimp; the latter, two kinds of sea weeds. The consumption of beef is only about 1.535 lbs. per annum, and of fresh milk per person per year less than a quart. Morimoto states that 2,089,731 dozens of cans of condensed milk were used in one year; but when one reckons the population of Japan as equivalent to 45,480,000 adults, this amount of condensed milk is insignificant. Other details concerning the Japanese diet will be found in Table XLI.

Kondo (1928) has reported on the dietaries of Kyoto citizens, and has tested the dietaries on experimental rats. The diets were found adequate as to energy, and contained about 12 per cent. of protein derived from polished rice and soy bean and some meat protein. He concludes that the diet is adequate in its vitamin content but that the inorganic moiety needs improvement. He notes that the consumption of milk is very small and that of alcoholic drinks relatively large. The diet is too low in protein considering that the quality is not high.

Infant Feeding in China and Other Oriental Countries.— It will be apparent from what has been said of the Oriental diet that the amount of milk available for children is very small. Since the food is not suited for the proper nutrition of young children, it has been the practice of mothers to nurse their children for very long periods, 4 or 5 years being not unusual. The early weaning of infants in Europe and America is possible because of the abundance of cow and goat milks. If the Orien-

tals weaned their infants at 9 to 12 months of age, their infant mortality would be greater than it is. The diet of the Oriental child the first few years after weaning is in certain respects less satisfactory than that of children of the Western Hemisphere. The Chinese, Japanese and Filipinos have not had a supply of

TABLE XLI

National Diet of Japanese People

	Consumption per man	
	Per year	*Per day*
Rice	5.32 bus.	.117 gals.
Barley (naked)	0.87 bus.	.019 gals.
Other grains	0.47 bus.	.010 gals.
Total (grains)	6.66 bus.	.146 gals.
Legumes	0.59 bus.	.102 gals.
Garden radishes	55.9 kg.	154 g.
Sweet potatoes	80.8 kg.	221 g.
White potatoes	15.4 kg.	41 g.
Other vegetables	56.7 kg.	155 g.
Total (vegetables)	208.8 kg.	571 g.
Fish	17.6 kg.	49 g.
Shell fish	2.2 kg.	6 g.
Seaweeds	1.2 kg.	3 g.
Other water products	1.8 kg.	5 g.
Total (water products)	22.8 kg.	63 g.
Beef and veal	1.58 lb.	1.9 g.
Pork, horse meat, mutton and goat meat	0.68 lb.	0.8 g.
Chicken	0.27 lb.	0.4 g.
Eggs (chicken and goose)	17.8 pieces	2.7 g.
Milk	1 qt.	....
Total (meat, fowl and milk)	.....	....
Fruits	13.5 kg.	37 g.
Sugar	14.1 lbs.	17 g.
Salt	39.6 lbs.	49 g.
Tea	0.7 kg.	1.9 g.
Alcoholic liquor	38 pints	0.44 gill
Tobacco	1.5 kg.	4.1 g.

Table taken from "The Standard of Living in Japan," by K. Morimoto, 1918

milk until recent years, when canned and powdered milks have been introduced.

Japanese children born in America have had a very different food supply from those in the mother country. Thirty years ago there was a great influx of "picture brides" into the Pacific coast states. We have therefore a large number of children in this country of Japanese parentage. Both boys and girls are larger at all ages than are Japanese children born and reared in Japan (Kanzaki, 1921). Without doubt it is the superior food which they have received which has made them outgrow their relatives across the Pacific. The question has been raised on several occasions when the size of a people has been mentioned in connection with the nature of their food, whether size is not entirely a racial characteristic and inherited, rather than determined by such agencies as nutrition. We have some very good evidence on this point in the histories of our experimental rats which is described in Chapter XXVI. When the nutrition of these animals fell just below a certain standard, there was no easily observable sign of malnutrition. Their appearance and fertility remained such that they could have been judged by any one to be "normal," yet the size diminished from generation to generation. The inferiority of successive generations may be the result of injury during the nursing period, or due to confining the young after weaning to the same inferior diet as that of the parents. The increase in size of Japanese children born in California and fed upon the products of its farms, over that of children of the same ages in Japan, harmonizes with this view. Horses introduced into the Shetland and Orkney Islands have degenerated in size, as have all other island horses. This is another illustration of the effects of a scanty and somewhat inadequate food supply upon animals. We are informed that Shetland ponies bred in America, and pastured on the rich fields of the middle western states, grow larger with each succeeding generation. Roxas (1922) says that the principle under discussion applies to the Filipino dietary. He suggests the importance of a greater and cheaper vegetable production, and a source of dairy products as a means of improving the nutrition of the Filipinos.

Diet of People in Labrador.—Appleton (1921) has contributed valuable data on the diet of the people of Labrador. Beriberi, scurvy, edema, and ophthalmia of dietary origin are of fairly common occurrence. She reported one case of pellagra.

A most interesting point about Appleton's report is that sometimes one and sometimes another of the deficiency diseases develops among people whose diet is similar in different households. The entire population is evidently in a state of nutritional instability, and small modifications in the diet determines which disease will appear. The table gives the approximate consumption of their principal articles of food.

TABLE XLII

Bolted wheat flour	1¼ to 1½ barrels per person per year
Salt meats, pork or beef	1 or 2 barrels for a family of eight
Salt codfish	2 to 4 quintals " " " " "
Salt herring	1 to 3 barrels " " " " "
Molasses	160 gallons " " " " "
Potatoes	1 to 2 barrels " " " " "
Rutabagas	1 to 2 barrels " " " " "
Dried peas	20 to 40 gallons " " " " "
Raisins	10 to 20 pounds " " " " "
Butter substitute	160 pounds " " " " "
Condensed or evaporated milk	From a few tins to two cases
Tea	20 to 40 pounds per year for family of eight

Meat, salt fish, potatoes and dried peas are eaten only once or twice a week; their supply is usually exhausted by April. Game is scarce. Fresh trout, cod and salmon are caught only during the summer. Gardening is little practiced, but in favorable seasons enough cabbage may be grown to last until November. A sack of onions may be added to the above list. Poultry are scarce, and egg powder is imported for making cakes for fêtes. The only fresh fruits eaten are partridge berries and a little yellow berry called locally "baked apple." Dock and Alexander plant which resemble spinach and parsley respectively could be used as greens, but they are little eaten. Cow's butter is used only by a few families. Many individuals drink 10 to 18 cups of tea a day.

Spruce beer made from young spruce tips is a popular beverage in the spring; it has anti-scorbutic value. Another interesting finding was that popular belief attributes curative value to raw birds' livers for night blindness. This liver would supply vitamin A. Dr. Appleton states that night blindness must be due to retinal fatigue, rather than an inflammation, which is the case with snow blindness and in which a photophobia is extreme. Poor diet may increase the possibility of seeing after-images just as fatigue does.

Diet of Italian People.—Mariotti (1919) has discussed the diet of the better class of Italians. Many kinds of cheeses are used in their cookery, and extreme care is taken to develop special flavors in foods; for example, strawberries are never eaten with cream, they are not washed in water but in wine, and they are served with claret or lemon juice and sugar. Peaches are soaked in white wine at the table. Sliced ham is served with fresh figs to bring out its flavor.

The peasantry live largely on cornmeal made into a mush or into a flat cake without yeast. The Tuscan and Umbrian peasantry are said to live almost exclusively upon a vegetable soup. Beet root, cabbage, broccoli, or potatoes are cut into small pieces and boiled in water to which is added a small slice of pork chopped very fine with an onion and some parsley. Tomato paste is added to this mixture. When served it has grated cheese sprinkled over it. It is said that only at Christmas and during the haying season do the peasantry drink wine or eat meat. No discussion of Italian food would be complete without mention of the macaroni. Macaroni is only one kind of what is called "pasta." There are many varieties of this food-stuff, of which the more familiar examples are spaghetti and vermicelli.

Davis and Wood (1921) also reported on the food of the Italians. They state that wheat, corn, and other cereals, vegetables, fruits, chickens, pigs, and the milk of the goat constitute the food of the farmers. Fruits and vegetables are produced in great quantities in Italy and even the poorest people have them in abundance. Children drink goat's milk, since every peasant has his goat, or else his neighbor has a supply. They consume little meat, and that which is used is made into stews, with macaroni and tomato sauce. Eggs are used more abundantly than meat. Desserts of the Italian are frequently fruits. The Italians make their own cheese from goat's milk. They are very fond of green vegetables, and in the large American cities Italians select in the markets a larger proportion of these when they can afford them than do other nationalities. The studies of Davis and Wood and of Gillett (1922) emphasize that Italians in their own country have an abundance of goat's milk, cheese, and green vegetables. In American cities they find milk and green vegetables expensive; if economy is necessary, they substitute coffee for milk. Since they have been accustomed to liberal amounts of Italian cheese, they buy this cheese at a dollar and a half a pound rather than American cheese at fifty cents a pound. A

taste for sweets is soon acquired in this country, and they also come to use the refined cereal products, since they are one of the cheapest foods. Gillett states, "No milk and too much coffee and candy are the greatest factors of the nutritional problem of the malnourished Italian children." Mudge (1923) has also described her studies on the Italian dietary.

TABLE XLIII

DISTRIBUTION OF FAMILIES ACCORDING TO THE AMOUNT OF CALCIUM RECEIVED
(0.67 gm. per man per day taken as a standard)

Number of grams of calcium per day per man	*Number of families receiving specified amount of calcium*		
	Italian	*Jewish*	*Negro*
Less than 0.5 gram	7	8	12
From 0.50 gm. to 0.67 gm.	13	7	5
From 0.67 gm. to 0.84 gm.	10	5	5
From 0.84 gm. to 1.00 gm.	7	2	3
From 1.00 to 1.25 gm.	4	0	1
1.25 gm. or more	3	0	1

DISTRIBUTION OF FAMILIES ACCORDING TO THE AMOUNT OF PHOSPHORUS RECEIVED
(1.32 gm. per man per day taken as a standard)

Grams of phosphorus per man per day	*Number of families receiving specific number of grams of phosphorus*		
	Italian	*Jewish*	*Negro*
Less than 1.0 gram	6	3	8
From 1.0 to 1.32 gram	7	6	8
From 1.32 to 2.0 grams	23	9	9
2.0 grams or more	8	4	2

Phillips and Howell (1920) reported an interesting paper on racial and other differences in dietary customs. They studied the Jewish, Italian, and Negro dietaries. The following menus are typical of each:

Jewish Menus.—Breakfast: Coffee with milk and sugar, rolls,

and sweet butter. Lunch: Rye bread, with either smoked salmon or kippered herring. Supper: Soup with vegetables and meat, potatoes, and rye bread.

Italian Menus.—Breakfast: Coffee with milk and sugar, and bread. Lunch: Soup made with vegetables, with small pieces of onion and pork, and Italian bread. Supper: Cod fish, cooked in oil, potatoes, Italian bread or spaghetti with tomato paste.

Negro Menus.—Breakfast: Pork chop, corn cakes, coffee, and condensed milk. Lunch: Fried hominy, syrup, and tea. Dinner: Fried ham, sweet potatoes, canned corn, bread, and butter.

In commenting upon these studies they stated that the diet of the Italian was characterized by a large amount of wheat products, green vegetables, and olive oil; the diet of the negro, by pork products and milled grains such as rice and hominy, whereas the Hebrew adhered more or less strictly to the dietary laws. An interesting feature of their studies was the calculation of the amounts of calcium, phosphorus, and iron received. These are shown in Table XII.

Sherman and Gillett found in a study of 92 families in New York City in 1917 that 48.9 per cent. of the families were receiving less than the standard allowance of 1.44 gm. of phosphorus per man per day; 53.2 per cent. were receiving less than 0.68 gm. of calcium and 41.3 per cent. were receiving less than 15 mg. of iron. The study in the winter of 1918 showed the following increased deficiency:

TABLE XLIV

	1918 *Per Cent.*	*1917* *Per Cent.*
Families receiving less than the standard allowance of phosphorus	61.8	48.9
Families receiving less than the standard allowance of calcium	57.3	53.2
Families receiving less than the standard allowance of iron	51.2	41.3

Phillips and Howell in commenting on these studies, say that doubtless this deficiency in mineral content was one of the causes for the undersize and lack of vigor noted among the children of the families studied. Among the Italian children there were many cases of rickets,—in fact, one would seldom see a child between 1 and 8 years of age who was not extremely bow legged. Sherman and Gillett found that in many homes no milk was bought because it was considered too expensive, but at the same

TABLE XLV

	Italian	*Jewish*	*Lithuanian*	*Negro*
Probable deficiencies	Calcium and vitamins	Iron and vitamins. In cases of financial pressure also calcium.	Iron and vitamins.	Calcium and vitamins.
One common dish	Beans, macaroni, tomato sauce and oil.	Gedemphe fleisch (pot roast made of brisket). Meat soaked according to dietary laws. Cooked with onions and seasoning in very small amount of water (in iron pot) for about 3 hours. Potatoes added one hour before time to serve.	Beef and pork, chopped fine, enclosed in a ball of dough 1 inch thick and boiled in salted water or soup 15 minutes.	Cush—leftover cornbread (soaked in water in which greens were cooked) fried with onions in pork fat.
Typical meals: Breakfast	White bread and coffee.	White bread or rolls; sweet butter, eggs or herring, potatoes, coffee. Milk for babies.	Rye bread and coffee.	Only two meals a day, and these whenever spirit moves.
Midday meal	Bread, fried peppers, eggs, ham or potatoes.	Smoked salmon, rye bread and butter, tea. (From the delicatessens.)	Soup, meat, potatoes, sauerkraut (or other sour food), rye bread, coffee or tea.	Fried pork, rice, soda biscuit, coffee.
Evening meal	Macaroni, beans, tomato sauce, oil.	Barley and mushroom soup, Gedemphe fleisch and potatoes, pickles or grated horse-radish, rye bread, tea. Compotè of dried fruit, sometimes cake made without butter or milk.	Meat, bread, tomatoes (perhaps cake, canned fruit), tea.	Greens, salt meat, cornbread, sweet potatoes, buttermilk.

TABLE XLV—*Continued*

	Italian	*Jewish*	*Lithuanian*	*Negro*
Striking characteristics (both desirable and undesirable)	Little meat. Large amount cheese and beans. Goat's milk in their own country—slow to use cow's milk here. Generous quantity of leafy vegetables, except when extreme economy is a factor. Few root vegetables or potatoes. Fruit if can be afforded. Oil—often a gallon a month. Much bread and macaroni—often 4-8 lbs. bread and 1 lb. macaroni daily. Strong prejudice against oatmeal and American cheese or oil. Much fried food. Taste for sweets acquired here.	Moderate amount of fresh meat and fresh-water fish. Large amount of smoked, salted, spiced beef, tongue or fish. Moderate amount of milk, cheese and eggs. Large quantities of legumes, potatoes, carrots and onions. Sorrel, beets, or beet greens in soup. Very few other green or fresh vegetables. Dried mushrooms. Dried fruit, conserves and preserves used freely. Fresh fruit between meals. White or rye bread and rolls. Coarse cereals in soup. Sour salts in soup. Sour cream and sweet butter. Very little fried food. Food generally over-seasoned, over-rich, over-sharp or over-concentrated.	Chief foods: Smoked or salted meat or fish (often 4 lbs. a day). Sour milk, buttermilk. Sour salts in soup and pickles. Beets, tomatoes, onions (fried), potatoes. Few green vegetables or cereals. Considerable fruit. Moderate amount of fat and sugar. Rye bread.	Chief foods: Pork. Cornbread (often 3 times a day). Molasses. Cabbage and turnip greens. Lard or bacon drippings. Potatoes—sweet and white (always fried). Buttermilk in soda biscuits. Butter substitutes. Little milk. Melons.

Table taken from "Studies on Racial Dietary Habits," by Lucy Gillett—*Jour. of Home Econ.*, 1922, vol. xiv, 261.

time a small piece of Italian cheese was purchased at a dollar and a quarter a pound.

Gillett (1922) has contrasted the dietaries of Italians, Jews, Lithuanians, and Negroes. She cites meals as well as discusses probable deficiencies and striking characteristics.

Dietary Study in Poland.—Morzkowska and McLaughlin (1928) have reported studies made in the fall of 1926 in Poland on Polish food habits. A Pole is more likely to begin the day with tea than with coffee. The staff of life among the peasants is rye bread; this is a coarse black bread depending upon wild yeasts for fermentation. In the cities a finer flour and cultivated yeasts are used. Baking powder is almost unknown. Several varieties of cereals play a large part in the diet, but not in the form of prepared breakfast foods. Barley, buckwheat, millet or wheat grists are made into thick porridges and sour soups, or are baked in the oven with salt pork and served with meats or milk.

A Pole eats about one-half as much meat as an American, but it must be remembered that peasants make up about 40 per cent. of the population, and that they eat meat, usually pork sausage, only on special occasions, such as weddings or holidays. In the country, game, especially partridges and wild hares, and smoked meats "jerked" over aromatic fumes, are favorite delicacies; in the cities, brain, liver, tripe, lungs, and tongue are preferred to muscle meats.

Potatoes are eaten extensively; they form part of almost every meal. Cabbage, especially as sauerkraut, is popular. Beets are used mostly for making soups. Barscz, made from fermented beets with the juice of fresh beets added to give a red color, is a favorite soup. Vegetables are cooked for a long time and are usually served in thick sauce. Fresh fruits, though less abundant, are the same as in the United States. Milk and eggs are used freely in the country, but the milk supply for the cities is not well organized. Salt pork, more than butter and lard, is used for fat. Butter is a valuable export. The per capita consumption of sugar is less than one-fifth of that in the United States.

Daily menus show that there is a marked preference for the flavor of lactic acid and other products of fermentation. Sour milk and cream are used in large quantities and fermented vegetables and grains appear on the daily menus almost constantly. The following are typical November menus of two classes of families whose dietary habits were studied:

MENU FOR A TYPICAL DAY IN A PEASANT FAMILY

Breakfast: Zur,* boiled potatoes, black bread.
Dinner: Sauerkraut soup with peas, potatoes, barley with milk, black bread.
Supper: Sour milk, boiled potatoes with salt pork, black bread.

MENU FOR TYPICAL DAY IN A SQUIRE'S FAMILY

Breakfast: Rye bread and butter, plum marmalade, raw smoked bacon, tea or cereal coffee with milk and sugar.
Dinner: Puree of vegetable soup, croutons, roast hare, mashed potatoes, beets, sauerkraut salad, baked apples, black coffee.
Supper: Smoked ham, bread and butter, rice and apple pudding, tea with sugar.

* Zur is a soup made from a fermented rye flour together with potatoes and sour milk or cream. (Recipe is given in full in the paper.)

The experimental facts presented in earlier chapters establish clearly that for animals the choice of food is of the greatest significance for growth, fertility, and longevity. The special difficulty of securing an adequate calcium supply, as well as an abundance of the several vitamins, emphasizes the element of safety which results from a proper selection of foods. It will be evident from what has been said of the dietaries of different primitive and less progressive peoples, that many of these have developed better physically, than have many people of to-day who are living in the industrial regions of the world. Their teeth were better, with few exceptions, than those of modern Europeans and Americans. They escaped rickets in regions where now white children frequently have the disease. Egyptian skulls of three thousand years ago show that all people who lived long ago did not have good teeth. In this particular region their teeth were of poor texture and did not last long. They suffered much from apical abscesses, and tooth decay was by no means uncommon. In seeking an explanation for this faulty dental structure we were led several years ago to suggest that the diet of the ancient Egyptians was not so good as that of many primitive peoples whose teeth were superior to theirs; an inquiry into the character of their food supply convinces one that this was true.

It has been pointed out on several occasions that the present tendency in the United States is to derive a relatively large part of the diet from refined cereal products, muscle meats, sugar, and potatoes,—a combination of foods which does not support satisfactory development, longevity and fertility in any animal with which experimental studies have been conducted. In dis-

cussing present-day dietary practices in America and their relation to defective nutrition, especially as shown by the prevalence of rickets, tooth decay, and malnutrition in children, it will be well to briefly discuss the changes in agriculture which have taken place in recent decades, since these are mainly responsible for the dietary changes which have been made.

Grass an Enemy to Agriculture.—Huntington pointed out that grass has always been the great enemy of agriculture. Primitive man could not compete with it, and accordingly could not practice agriculture extensively. The clam shell and the crooked stick were an ineffective armament for the early agriculturalist. When draft animals were domesticated, he was capable of eradicating grass, but the great labor involved in harvesting and threshing cereals made their cultivation on a large scale difficult. Egypt was an especially suitable place for the growing of cereals with primitive implements, since the flooding of the land by the Nile each year prevented the formation of the tough sod which prevailed on grassy plains; it was for this reason that grains were grown there in large quantities from very early times.

Cereal grains may be kept in a dry state for years without deterioration. They served as a reserve food supply to be drawn upon during the less hospitable parts of the year, and were a safeguard against famine. Joseph put away cereals in expectation of lean years. However, the amount of cereals eaten by many primitive peoples was relatively small. The Indians grew corn, but when it was harvested the amount often sufficed for little more than a great feast. The cereal grains at the present time furnish 35 to 45 per cent. of the food supply in parts of Europe and America; this is an innovation in human experience. More and more cereals, meats of the muscle type, tubers, and sugar have become the prominent articles of diet during recent years.

Importance of Steel-faced Plough to Agriculture.—It was never possible to deal effectively with grass sod in farming until the invention of the modern steel-faced plough. This invention was an American one, dating about 1840. This type of plough cuts a ribbon of soil about a foot wide and three inches or more in depth, and turns it over with precision, exposing the roots of the grass to the drying action of the air. In this way it is comparatively easy to kill the grass in the toughest sod. The plough of the type of the sharp stick which scratched the earth

and tore it up in a rough, irregular manner was an Aryan invention and is very old. The modern plough made possible the conversion within a few decades of the vast prairies of the United States and Canada, of Argentina, Australia, Southern Russia, Roumania, and other places into wheat or other grain fields. The world's production of cereals during the last seventy-five years has increased greatly with each succeeding decade, so that a greater proportion of the human diet can now be derived from this source than ever before.

Steam Transportation and Cereal Production.—The development of steam transportation in the second half of the nineteenth century was another factor of the greatest importance in stimulating cereal production and cereal consumption during the last 50 years. The railroad and steamship make it possible to transport grain from any part of the world where it can be produced economically to the great centers of population in the industrial areas. Without this transportation it never would have been profitable to extend cereal cultivation to the proportions it has reached. With the perfected agricultural implements such as the plough, the reaper, the binder, and the thresher, which make for economy in grain growing, and with cheap long-distance transportation making a world market, the time necessary for the marketing of grains is so long that the problem of spoilage of flour became a very important one. For this reason inventive genius was turned toward solving the problem of milling a flour which would keep better than the whole ground kernel which had been produced through human history between the upper and nether millstones. This problem was effectively solved in 1879 when the modern roller mill process of milling wheat was perfected. In this process, the kernel is not ground but is broken between rollers; the germ, bran, and coarser grades of flour can be mechanically separated. That part of the kernel which has physical properties which make it crush between rollers so finely that it will pass through bolting cloth, is marketed as the most desirable in the eyes of the manufacturer and distributor.

The appetizing qualities of meats are sufficient to insure for them a regular place in the diet of man wherever they can be afforded. The profit in sugar production has led to an enormous increase in sugar consumption in the United States and elsewhere. The consumption in this country per person per year is about nine times what it was a century ago. It is now about one hundred and fifteen pounds per capita per year.

These facts are sufficient to convince one that the dietary practice of Americans and many Europeans have changed greatly within a few decades. We have been trying an experiment in human nutrition on a nation-wide scale, with a type of dietary which no people in history ever tried to live upon before. There was no way in which the results of such an experiment could have been foretold, for the dietary properties of individual foods were not understood, and the nutritive requirements of the body were essentially only half understood. It is possible, however, after approximately two generations of experience with a diet of the white bread, meat, sugar and potato type, with small, but generally inadequate additions of other foods of kinds which are capable of correcting the defects of the principle components of the food supply, to attribute certain unfortunate effects definitely to an unsatisfactory dietary. All the information available seems to warrant the conclusion that we may attribute in great measure the high incidence of malnutrition among children of pre-school ages, the faulty bone growth, bad teeth and faulty posture, to inadequacies in our national dietary, and to preverted appetites which result from pampering and the development of a preference for sweet foods.

CHAPTER XXVIII

THE DIET IN RELATION TO THE TEETH

Prevention of Tooth Decay.—There is no subject of greater importance to human welfare than the preservation of the teeth. A score of ailments may result from the introduction of bacteria or their poisonous products into the body through decayed teeth, or through diseased areas surrounding the necks of the teeth. There has been much discussion among dentists and others concerning the cause of tooth decay, but as yet there is no unanimity of opinion concerning it. In caries, the enamel becomes disintegrated and breaks down in a manner similar to what we should expect if acid were constantly applied to the surface of the tooth at one point. When a cavity is formed, food particles and mouth secretions accumulate within it. It becomes the focus of fermentation with further production of acid. Several questions present themselves, viz.: Are the bacteria the primary cause of the carious cavity? Are there special kinds of bacteria which produce caries? Why do some persons escape caries while others develop it? Why is caries more frequent in early life than in later life? Why are caries and pyorrhea seldom found in the same mouth? Are there differences in the structure of the teeth which make some immune and others susceptible to caries? Is there a relation between diet and tooth decay? Has the composition of the saliva anything to do with dental caries? These are only a few of the questions which have been raised in recent years by those who are thinking critically about all the facts which observation has yielded, or who are experimenting in the field of preventive medicine.

Since bacteria are always demonstrable in the dentinal tubules in a carious cavity, many have believed that caries is essentially a bacterial disease, that its prevention lies in mouth hygiene, and that the preservation of the teeth is to be sought in cleanliness and the filling of cavities while they are small. The attitude of the United States Public Health Service may be taken as

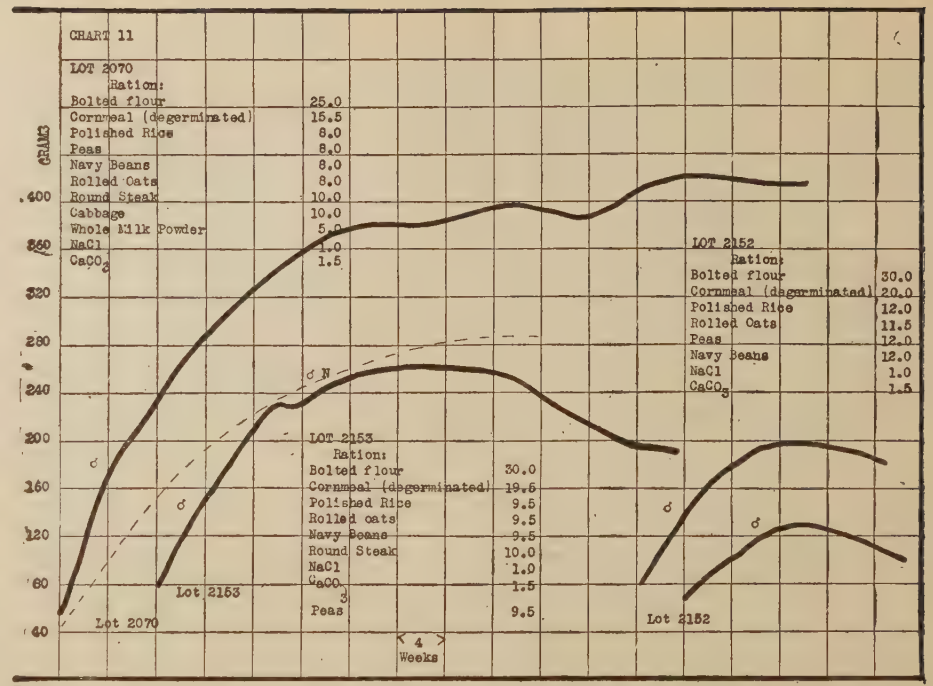


CHART XI.—The three curves shown illustrate the lack of vitamin A and the low quality of the proteins in a mixture of milled cereal products and legume seeds. In each experiment the chief mineral deficiencies were corrected by the addition of sodium chlorid and calcium carbonate. Lot 2152 had a diet which contained proteins of poor quality and was deficient in vitamin A. Note that the animals grew but little, and began very early to fail, as shown by loss of weight. Their entire lives were not longer than the period during which a well-fed rat normally grows.

Lot 2153 had a diet essentially like that of Lot 2152 except that its proteins were enhanced by the inclusion of round steak. The diet was still deficient in vitamin A although by no means lacking in it. Growth was rapid for a time but the full adult size was never reached, and the animals early developed the characteristics of old age.

Lot 2070 had essentially the same diet as Lot 2153 except that cabbage and whole milk powder (Merrill-Soule) were included. These served to further enhance the proteins and to furnish vitamin A in moderate amount. The result is very spectacular. The animals grew rapidly to large size and maintained for a long period the characteristics of youth.

representative of that of nearly all who have become active in promoting preventive dentistry. Clark and Butler (1920) stated that "Dental caries is caused by the action of bacteria or germs which normally inhabit the mouth. These germs, acting in the presence of food débris and certain elements in the saliva, result in the formation of an acid which attacks the enamel covering the exposed parts of the tooth, after which underlying softer parts become rapidly destroyed. Many other factors are actual and potential causes of dental decay and its progress, such as (1) low resistance of the teeth to decay because of developmental defect (antenatal and postnatal); (2) faulty diet (both of the mother during pregnancy and of the child); (3) neglect of dental attention through ignorance of the parents; (4) the cost of dental attention, a serious consideration with families of low economic status; (5) failure of the child to call attention to the condition of the teeth, either because it is too young or because of fear; (6) lack of dental facilities, so common in rural sections." It is pointed out by these authors that a very high percentage of undernourished children show marked evidence of dental decay. They cite the results of the examination of 270 children by the United States Public Health Service, which showed that 33 per cent. of these had from 1 to 4 cavities, 48 per cent. had from 4 to 8, and some had 9, 10, and 11 cavities. This excellent paper fails to emphasize the importance of including in the campaign for the preservation of the teeth an effort to change the character of the nation's diet, in order that developmental defects shall grow less common and less severe as time passes. Emphasis is laid entirely upon the importance of the dental hygienists, who limit their work to cleaning and polishing the surfaces of the teeth above the gum margins, to the dental clinic for the repair of the teeth already in a state of decay, and to the teaching of mouth hygiene. Such a program is based upon the belief that cleanliness of the oral cavity is of first importance as a preventive measure for preserving the teeth.

Certain facts have been brought to light by experiments on animals which cast doubt on whether the above quotation takes into account all the essentials of an effective program of preventive dentistry. It does not mention any possible means of preventing caries through improving the structure and position of the teeth. Not all authorities are convinced that the above program is sufficient to accomplish the desired result of making the nation's teeth sound. An eminent dentist recently made the

assertion that most of the sound teeth in the world existed before the tooth brush was invented.

In England and in America we are in a stage of progress where repair and cleanliness are goals toward which school authorities and parents are being urged to work. This is essentially trying to put an attractive superstructure upon a faulty foundation. In its present form this movement for the preservation of teeth is founded upon a plan which will call for more and ever more investment of time, money and human effort without any prospect of relief from the burden. The senior author pointed out several years ago that *the only prospect of relief from the present deplorable condition of the teeth in America was through giving attention to the problem of forming sound teeth during the period of growth.* He stated that this involves *the application of scientific nutrition to the regimen of the expectant and nursing mother and to the management of infants and children, and stated that without satisfactory prenatal nutrition the teeth of the child will be faulty.* This idea has been accepted by almost all dentists, and is now being emphasized as an outstanding feature of the campaign against tooth defects.

Importance of Diet During Prenatal Life.—The importance of the diet is emphasized by the fact that the calcification of the first set of teeth is said to begin between the fourth and fifth months of pregnancy and the calcification of the first permanent molars shows a tremendous increase in the last third of prenatal life. It is clear that good teeth are determined, in great measure, before the child is born. It is true that serious damage may, and often does, occur to the teeth in postnatal life. The infectious disease of childhood such as measles, scarlet fever, etc., may temporarily interrupt the development of the teeth, and when development is resumed a fault may be visible in the enamel to mark the event.

The researches of the last few years which have shown so clearly the possibility of influencing favorably or adversely the structure and quality of the bones through the character of the food supply have led investigators to question whether there may not be an important dietetic factor operating in determining the poor quality of the teeth of modern Americans and Europeans. No other nations have so great an incidence of tooth defects and dental diseases, and of diseases involving the attaching tissues of the teeth. During the last decade the widespread interest in the nutrition of children of all ages has resulted in the accumula-

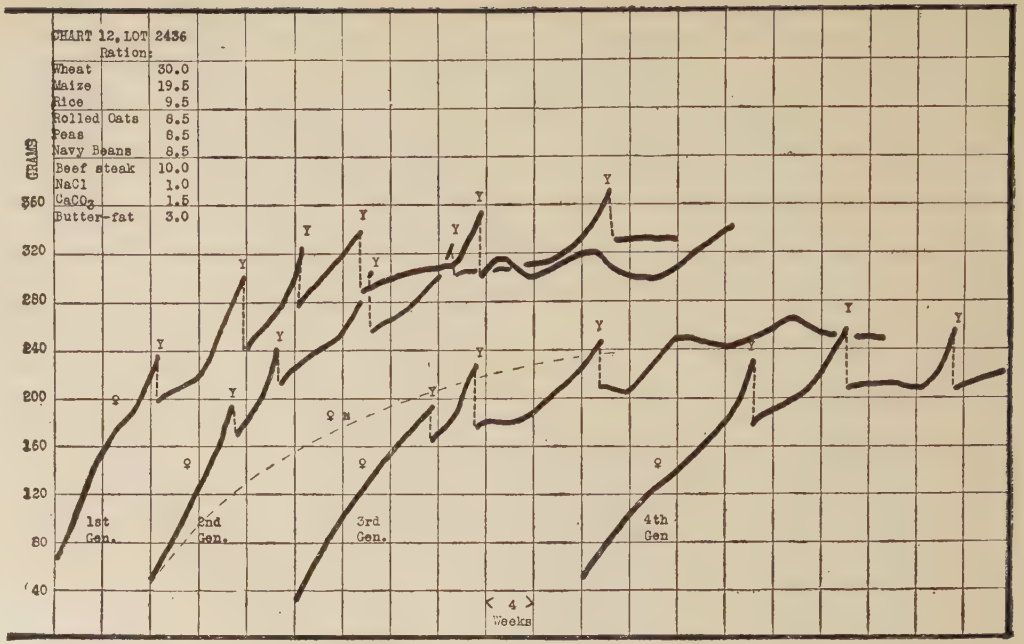


CHART XII.—This chart shows how good growth and high fertility may be secured in rats on a monotonous diet consisting of cereal grains, legume seeds and beefsteak supplemented with calcium, sodium and chlorine and with a source of vitamin A. If the inorganic additions are omitted the animals on this diet would be entirely unable to grow. If the butter fat were omitted they would be very inferior, and succeeding generations would be of low vitality. This would also tend markedly to reduce the fertility. If the beefsteak was omitted and no other supplementary source of protein was added in its place the animals would again be stunted, especially in the second generation. The omission of a supplementary source of protein would affect in a marked manner the capacity to produce and rear young. With the diet as tabulated, however, we have a complete food mixture and the family took it without any variation through successive generations without any striking deterioration in vitality.

tion of a large number of weight-height-age records which, together with the clinical observation on skeletal defects, defective musculature, anemia, faulty posture, and other evidences of malnutrition, reveal that a large percentage of our children are not properly developed, and that in many cases this is due to lack of a properly planned dietary. One would suspect that dietary defects which cause such marked inferiority of bones would likewise cause structural defects in the teeth, and that these defects might make the teeth susceptible to disease. Several efforts have been made to throw light on the many questions relating to dental problems by experimental work directed toward determining the effect of faulty diets in animals on the structure and health of the teeth.

Teeth of Primitive Peoples.—Weld (1795) in his book on "Manners and Customs in Philadelphia" stated that American-born men and women had decayed teeth at an early age. He thought this was due to the very general use of salted provisions. He eliminated climate as a causative agent, since the Negroes and Indians had sound teeth.

Catlin (1861) in his book "The Breath of Life" stated that it was a common custom among the Sioux tribes to collect the skulls after the scaffolds gave way on which the bodies had rested, and to preserve them in large circles on the ground. Catlin saw many of these and says: "I was forcibly struck with the almost incredibly small proportion of crania of children; and even more so, in the exceptional completeness and soundness (and total absence of malformation) of their beautiful sets of teeth, of all ages, which were scrupulously kept together, by the lower jaws being attached to the other bones of the head." "The teeth arrange themselves very regularly as the keys of a piano. Their teeth do not decay but preserve their soundness and enamel and powers of mastication to old age." He further stated: "The chief (of the party of 14 Ioway Indians who visited London with Catlin) informed me that one of the most striking peculiarities which all Indian tribes discovered amongst the white people was the derangement and absence of their teeth, and which they believed were destroyed by the number of lies passing over them."

Hrdlička (1908) reported that among Indians of the southwestern United States "Dental caries is not rare, though much less frequent than among the whites. It is usually a molar or premolar which is affected. Occasionally the gums or alveoli be-

come involved, resulted in suppuration. Necrosis of importance was not seen in the living nor in the bones examined. Defects of the palate in an Indian of full blood have not been met with, and but one minor case of hare lip came to notice."

Hrdlička (personal communication) states that at the National Museum there is a large collection of Indian skulls and bones from the Eskimos of northwestern Greenland; these bones are remarkable for their good development and solidity. The teeth of the Eskimos as a whole, including those of northwestern Greenland, are probably the best of any large group of people living to-day.

It is said that the people of the Sahara keep their teeth white by rubbing them twice a day with the inner bark of the walnut tree. Diseases of the teeth are said to be virtually unheard of, in spite of the fact that they eat a large amount of dates containing a high percentage of sugar.

Ottofy (1908, quoted by Howe) examined the teeth of Igorot children in the Philippine Islands and found that 68 per cent. of them had perfect teeth; the imperfections in the remaining 32 per cent. were so slight that they would have escaped notice if casually inspected.

Wells (1919, quoted by Howe, 1920) reported that the Highlanders of Scotland are comparatively free from dental decay, but the people living in the Lowlands have much decay. Speaking of the excellent dental condition of the Highland Scotch he says, "This is largely accounted for by their simple diet of natural foods." On the other hand he says of the extremely poor dental condition of the Lowlanders, "The great reason for this, to my mind, is the diet of more refined foods." Howe remarks that every dentist is familiar with the fact that many of the Swedish girls who come to this country as domestics have excellent teeth when they arrive, but after eating our refined and cold storage foods their teeth decay rapidly.

Ruffer (1921) stated that abnormalities of dentition are not peculiar to modern times either in France, England or Egypt. In England prehistoric remains have been found which show great abnormalities in the position of the teeth. Caries in teeth from ancient remains have been discovered in so many places that it is legitimate to doubt whether there was ever an epoch in these countries when the human species was not cursed by toothache.

From the earliest time caries has occurred in human teeth in

Nubia and in Upper and Lower Egypt. Ruffer quotes from an investigator who stated that both in Nubia and Egypt the ordinary form of caries is exceedingly rare, in predynastic and protodynastic people, and among the poorer classes it has never been common at all until modern times. . . . Dental caries became common as soon as the people learned luxury. The Hearst expedition excavated at the Giza pyramids more than five hundred skeletons of the aristocrats of the time of the pyramid builders; tartar formation, dental caries and alveolar abscesses were at least as common in these bodies as they are in modern Europe to-day, and at every subsequent period in Egyptian history one finds the same thing—the wide prevalence of dental diseases among the wealthy people of luxurious diet and the relative freedom from it among the poorer people who lived mainly on a coarse, uncooked diet.

Ruffer comments on the fact that in Egyptian cemeteries diseased teeth almost dropping out of abscessed cavities are very frequent. There were no teeth filled with gold or any other metal. The only set of artificial teeth was found in a Roman grave.

McClendon, Riley and Elwell (1923) reported that Sullivan and Chappel examined prehistoric Hawaiian skulls and found nearly all belonging to individuals over 40 years to show gingival caries of the teeth. They found caries in persons buried prior to 1778, a date which establishes that these people had had no contact with white people. The principal food of the Hawaiians was poi, made from taro root; since the calcium and phosphorus of this food are very low, they attribute the faulty teeth to its excessive use.

Teeth of an Island Community.—Marshall, Surgeon to the *Discovery*, visited the Island of Tristan da Cunha in 1926. This island is situated in the South Atlantic about two thousand miles west of the Cape of Good Hope and about four thousand miles northeast of Cape Horn, and lies somewhat north of a line drawn between the two Capes. At that time the population consisted of 75 men and 66 women. Sixty-nine were adults, 21 were between the ages of fourteen and twenty-one, and 51 under fourteen years of age. Three persons were over eighty, all women; the oldest man was sixty-eight. This island has been visited by ships only at rare intervals for many years, so the islanders are limited essentially to a local food supply. The appearance of the people suggested a satisfactory nutritive state. They have had few

epidemics of disease, and complain that after ships call epidemics of "colds" run through the island, lasting about three weeks.

The people possess cattle and sheep, some donkeys and pigs, and some hundreds of fowls and geese. There were 245 plots of land, varying in size from 20 to 40 yards square, planted with potatoes. Fish are plentiful and easily caught, but are not popular as a food. Hens' eggs are scarce and are consumed mostly by the wealthier families. Mollymauk eggs are eaten in large quantities, and some thirty thousand penguin eggs are collected annually. Young mollymauks are eaten in large quantities. Milk is nearly always plentiful, but for long periods the islanders are without tea, coffee, and sugar. Butter, jam, and marmalade are only very occasionally available; the inhabitants eat no bread, since wheat is not grown. The main source of carbohydrate is potatoes. Onions, pumpkins, turnips, carrots, beet root, and leeks are grown, but in small quantities. The outstanding shortages are flour, sugar, jam, and tea.

Out of 50 persons examined, only 6 admitted that they suffered from indigestion or flatulence, and these cases were of some years standing. The teeth of more than one-third of the islanders were examined for dental caries; the results were tabulated as follows:

Age Period:	3-14	15-20	21-44	45-90
Number examined	13	8	21	12
Number of teeth	290	235	662	337
Number of carious teeth	2	0	11	21
Number of carious mouths	1	0	5	8
Number of teeth missing	0	0	6	45
Percentage of carious teeth	0.68	0	1.6	6.2
Percentage of carious mouths	7.6	0	23.8	66.6
Chronic general peridontitis	0	3	14	7

Thus the incidence of dental caries is remarkably low, and would probably be still lower, if the entire community could have been examined. The teeth examined were almost without exception regular and well-shaped. Although in no case did anyone admit cleaning his or her teeth more often than once a week, and then with finger and soap, the teeth were clean and remarkably free from salivary calculi. It is probably correct to say that the islanders, as a whole, never clean their teeth. Chronic general peridontitis was present in various stages in no less than 58.5 per cent. of those examined between the ages of fifteen and ninety. This is probably not a disease of recent origin in the island, since histories, dating in some cases more than twenty

years back, were given of teeth becoming loose and falling out without showing signs of caries. How far the shortage of sugar and jam has prevented the spread of caries, and what influence the large consumption of potatoes, on which the islanders rely almost exclusively for their carbohydrates, has had on the incidence of chronic general peridonitis, are matters for discussion.

Cilento (1928) reports a most interesting contrast to the experience of the people of Tristan da Cunha, in that of the peoples of the Western Islands of the Territory of New Guinea. These islands formerly supported a numerous population of vigorous peoples, but commercial grants made to foreign companies during the past fifty years have deprived the natives of the best agricultural lands and they have been forced to subsist mainly upon "fula" or swamp taro, a starchy root, with a kind of yam "ura," crude sago, three varieties of breadfruit and two kinds of bananas as secondary food-stuffs. Some shark flesh, with the occasional addition of fish, pigeons, parrots and lizards, raise the protein moiety to the subsistence level. The diet is very poor in quality as respects protein, all vitamins, and some mineral elements, especially calcium. Dental defects are very common, and the degree of dental deficiency recorded was found to vary in different islands directly as the insufficiency of food as judged by quality. Cilento states, "In many of the Pacific islands, especially among the Polynesians, and contrary to the casual impressions of the visitor, early loss of teeth is a very marked characteristic of the native inhabitants, and one which existed to some extent prior to the arrival of Europeans. Where vitiating diseases have been introduced the process has naturally been accelerated." Loose, blackened and filthy teeth are extremely common among these islanders. The incidence of tuberculosis is also very high. Malaria is prevalent and makes it impossible to determine in how far disease and diet respectively affect adversely the structure and health of the teeth.

Teeth of American Children.—Rypins (1923) examined 1197 pre-school children of Kansas City, Missouri, between the ages of three and six years; of these, 27.2 per cent. had dental caries. There was over one decayed tooth for each mouth, and almost four decayed teeth for each of the carious mouths. Of the teeth erupted 6.3 per cent. were carious. Since these children were about ready to begin their schooling, these findings represent definitely the condition they present upon entering school. Once

in school, absence from studies is a handicap to the pupil and an economic loss to the community. On the basis alone of effects associated with dental caries such as toothache, abscesses, or time required for receiving dental attention, the problem is an important one. Considered in the light of consequences of major handicaps that can and do result from dental diseases,—rheumatism (often called growing pains), heart disease, and maldevelopment—the problem is a distinctly serious one. Dental disease therefore plays a serious rôle in the lives of little school

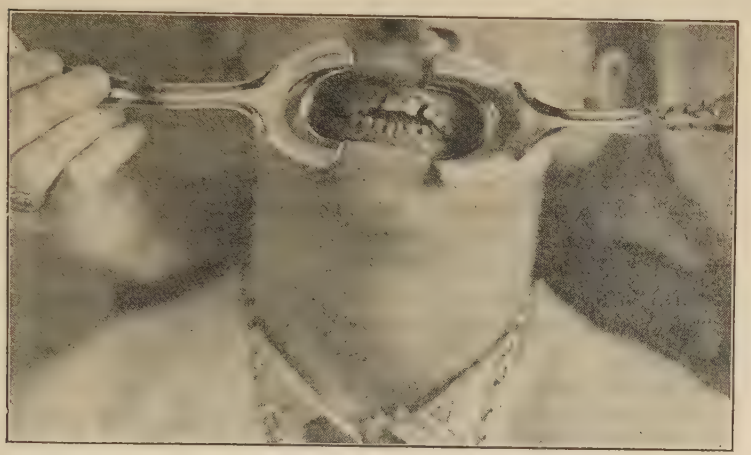


Fig. 34.—Illustrates the extent to which the teeth have degenerated among Americans in recent times. The trouble lies in great measure in faulty nutrition during prenatal life, infancy and childhood. A satisfactory diet is essential for the development of a sound set of teeth. If the jaws and teeth are poorly developed they must throughout life be vulnerable to bacteria agencies.—Courtesy of The Forsythe Dental Clinic.

children and the economic consequences are obviously of importance.

Cross (1923) stated: "Investigation has shown that 96 per cent. of the children coming to this country from the southern part of Europe have sound teeth, while it is well known that the teeth of 96 per cent. of the American children are defective. Strange to say, too, the vast majority of the foreign children never knew what a tooth brush looked like until they entered American schools." He further stated that all adult dental diseases except pyorrhea are the result of poor tooth development or neglect of the proper diet and hygiene during childhood.

Dr. P. S. Schenck (personal communication) gave us the fol-

lowing information relative to the condition of the teeth of white and colored children of Norfolk, Virginia. Their free dental clinics show that a very much smaller percentage of negro children have dental defects, and also that a very much smaller percentage of negroes are treated in the clinic. As soon as the negro child can take solid food, it eats quantities of greens, cabbage, turnip salad, and vegetables; it does not have the sugar and fancy foods which the average white child eats. Compared with the white child, the diet of the negro child contains a larger percentage of iron, calcium and other elements necessary for tooth development.

Dr. J. A. Murphy (personal communication) reported the following data concerning the condition of the teeth of the white and colored pupils in the public schools of Washington, D. C., during the years 1916-1923. There were 47,744 white and 27,968 colored pupils; 39,828 or 83.4 per cent. of the white, and 24,705, or 88.3 per cent. of the colored children were found to have carious teeth. The average number of cavities in temporary teeth of white pupils was 0.6 as compared with 1.0 for the colored. The average number of cavities in the permanent teeth was 1.3 for the white and 2.3 for the colored.

Dr. D. J. MacDonald (personal communication), who is familiar with the Island of Lewis, Scotland, states that the inhabitants for the most part are crofter fishermen, i.e., they cultivate a certain amount of land and also engage in fishing. Until about 50 years ago their diet consisted mainly of bread and porridge made from coarsely ground oatmeal and barley meal, and potatoes, fish, eggs, and milk. Since then, however, the consumption of white bread, tea, and other articles of "cultivated diet" has greatly increased, and although oatmeal is still very largely consumed, it is no longer the coarsely ground, home-milled variety, for it is chiefly imported. Except in the town areas, even at the present time, very little meat is consumed. The older people in the country districts have extraordinarily sound teeth and seldom require the services of a dentist; the same cannot be said of the younger generation, although they compare favorably with young people in Glasgow.

Dental Disease in Olmstead County, Minnesota.—Mebane (1924) reported on the health condition of about three thousand children of Olmstead County, Minnesota. In 50 children with severe chest deformities (rachitic) the permanent teeth had

erupted within the limits of normal variation. Dental caries is the most common defect; under-weight and tonsillar disease were next. Dental caries is more common in city children, and these children were more often under-weight. Malocclusion was found in 59 per cent. of the children examined, and in 40 per cent. the biting edges were irregular and often resembled Hutchinson's teeth. Five and one-tenth per cent. had rachitic deformities of the chest, and 12 per cent. had flat feet.

Mebane believes that teeth decay more rapidly during and after febrile diseases, due to the fact that during illness the teeth are not likely to be kept clean, and that elevation of temperature causes any residual food upon the teeth to decay more quickly and form a nucleus for beginning caries. He emphasizes the importance of the six year molars, in that they are the keystone of normal occlusion in the mouth and therefore their preservation is essential. When first erupting they often present deep incisura, which retain food easily; early decay along these crevices is common, and for this reason he emphasizes cleanliness.

Condition of Children's Teeth in Rochester, Minnesota.—Kappes (1928) during an examination of school children of Rochester, Minnesota, found two contrasting groups in the age range of five to thirteen years. The first group included children whose teeth were free from caries, and had required little or no dental work. The second group included those whose teeth showed caries involving half or more of the teeth. Children whose teeth showed ridges and defective enamel recognized as due to definite nutritional disturbances were not considered. Detailed inquiries were made as to maternal conditions during pregnancy, general health factors, past illnesses, environmental features, and particularly the details of the daily diet. The only feature that seemed to be of definite etiological significance in preventing decay was a diet composed largely of fruits and vegetables. She concluded that heredity, infectious diseases and the care of the teeth appeared to be of little or no significance.

Teeth of Italian Immigrants.—For many years Howe observed the teeth of immigrants arriving in Boston. He found the parents and children to have teeth far superior to those usually seen in Americans of the same age. Children born of these parents in America, when they reached ages comparable to those of their older brothers and sisters on arrival, had teeth

much poorer and more subject to decay. The teeth of the older children in America also deteriorated much more rapidly than those of children of Italy in the regions from whence they came. Similar conditions prevail in immigrants from rural Ireland and Scandinavia. Many Italian children in America suffer from rickets, whereas those living under the conditions of their native country do not. Their housing conditions have changed. In their native land they lived out-of-doors during the greater part of the year. In the large cities they are living in the tenements with a consequent lack of fresh air and sunshine, and with no place for the children to play but the noisy, dirty street.

Preservation of Teeth.—For several years an extensive mouth hygiene program was carried out by Dr. Fones in the schools of Bridgeport, Conn. He emphasizes the importance of whole cereal grains in the place of refined cereals, of brown sugar in place of granulated sugar, and stresses the importance of oral mouth hygiene. He is in accord with McCollum in the view that if teeth are given care and children take a proper diet much can be done to improve the health. He likewise stresses the importance of the diet of the pregnant mother and states that without satisfactory prenatal nutrition the teeth of the child will be faulty.

Hellman (1923) expressed the view that acceleration of body growth has an adverse influence on the process of dentition. This is certainly not true of either domestic or laboratory animals.

Bunting (1924) discussed the influence of prenatal feeding. A woman aged twenty-five years vomited frequently for 13 months, during which time she became pregnant and carried a child to full term. Although she was unable to retain solid food and became emaciated, the child at birth weighed 7.5 lbs. He seemed perfectly developed and thrived under splendid postnatal care. When he was 4 years of age he had a perfect set of deciduous teeth well arranged, on fully developed arches, without any signs of dental caries. This mother, a primapara, prior to her pregnancy had no dental caries, but later developed several cavities. It would seem that the mother had suffered more with respect to teeth than did the child. Bunting states that although we cannot say that the child's teeth cannot be affected by nutritional faults of the mother during prenatal life, it is quite within the limits of possibility. Although prominent defects resulting from nutritional disturbances are not apparent in the deciduous

teeth, stigmata may be produced on the permanent dental follicles which will influence later tooth development.

Views on Etiology of Caries.—Many views have been expressed concerning the etiology of dental caries. Rumsey (1921) believed diet to be an important factor in the etiology of caries. Seccombe (1921) stated that the essential factor in the formation of dental caries was the presence of carbohydrates in the diet. He found salivary stasis accompanied by fermentation to be present with caries. A diet containing an excess of acid-forming elements he believed to predispose to caries. He attributed caries without salivary calculi to over-ingestion of sugar. Indigestion, lack of mastication, or improper diet he stated may give rise to a thickened saliva containing an excess of mucin. He believes over-ingestion, auto-intoxication or trauma may result in gingivitis.

Mummery (1922) has given an extensive discussion of existing knowledge in regard to variations in the composition and structure of enamel, and their relationship to caries. He also gives an historical account of researches planned to produce artificial caries.

Bunting (1925) expresses the view that diet and nutritional disturbances influence dental caries by alteration of the oral environment rather than by production of structural changes in the teeth. Bunting, Nickerson and Hard (1926) report a survey of 427 patients showing a relation between the occurrence of dental caries and the presence of B. acidophilus. The latter is believed to be the etiological factor in caries.

Bunting (1928) has presented much further evidence of the relation between B. acidophilus and dental decay. Marshall (1928) gives an interesting discussion of the control of dental caries by means of diet. He reports that rats on diet deficient in vitamin A develop lesions similar to dental caries apparently only after maturity has been reached. The lesions so produced were more like those found in adult man than in the child or adolescent. In more advanced cases, pulp lesions have been demonstrated, such as abscess and pulp calcification, both of which occurred in teeth with and without caries. Although the growth curves, for the experimental animals, were typical for avitaminosis, all the animals did not respond to the same degree to these deficient diets. The lesions which have been demonstrated in rats were entirely dissimilar to those previously reported on dogs, for neither enamel nor dentin hypoplasia has

been found in the rat. Marshall doubts whether dental caries will ever be entirely controlled; he believes it is a problem in which diet plays only a part. The local conditions as controlled by saliva and as modified by the oral flora, he believes, are important factors in the etiology.

Experimental Studies on Dental Problems.—Howe and Keniston (1918) stated: "It would appear that the saliva of persons with teeth immune to caries varies, as does also the saliva of persons with carious teeth; that saliva may neutralize substances taken into the mouth, and that the average immune mouth has the greater power of neutralization; but the ratio of resting and activated saliva in immune mouths does not vary enough from that of carious mouths to prove that this ratio is indicative of the production and maintenance of immunity from caries in any individual."

Miller (quoted by Howe, 1920) believed that the fermentation of carbohydrate with the formation of lactic acid is the cause of tooth decay. Teeth were placed in a fermenting mixture of bread and saliva which was renewed from time to time so that it might not become alkaline. After 3 months there were defects in the teeth which could not be told macroscopically or microscopically from tooth decay. Bacteriologically his work was limited and he found no specific organism as the etiological factor for caries.

Howe (1920) repeated and extended this experiment. Teeth were placed in fermenting mixtures composed of maltose, lactose, saccharose, dextrin, white flour and bread. In some of the tubes saliva from individuals that had had extensive tooth decay were used, in others saliva from cases of no decay, and in others saliva from mixed cases. After 6 months some of the teeth showed an etched condition, some a decalcified effect, and in others no change. The effects produced were in general similar to those produced on teeth which had been subjected to a weak decalcifying agent. Howe comments on the pronounced differences of various teeth in their ability to withstand the effect of different decalcifying agents.

Howe fed guinea pigs on diets containing large amounts of starches and sugars in order to see whether or not he could produce tooth decay in animals by fermentation. The animals were on the diets 6 months to one year. The sugars were constantly in contact with the tooth surfaces. The flora of the mouth

became aciduric in character, but no effect on the teeth could be detected. For 3 or 4 months micro-organisms were fed which had been isolated from caries. The organisms were constantly present in the guinea pigs' mouths, but no effect upon the teeth could be demonstrated. Howe has produced rather extensive effects in the teeth, the alveolar processes, and jaws, by feeding guinea pigs vitamin-deficient diets.

Howe (1921) demonstrated that when guinea pigs were fed a diet consisting almost wholly of fat-free milk and rolled oats, decalcified areas are produced in the teeth, alveolar processes, bones of the skull, and other bones of the body. This diet would produce scurvy in a guinea pig. Recalcification was produced when the animals were returned to a normal diet. In a later paper (1922) he analyzed the teeth of normal guinea pigs and of pigs fed a scorbutic diet. The scorbutic diet produced extensive decalcification of the teeth and some parts of the bones. Calcification occurred as the result of adding vitamin C to the diet. Grieves (1922) reported the results of feeding rats various types of diets. Although there appeared to be no particular dietary deficiency associated with the occurrence of dental caries, he believed that a proper calcium to phosphorus relationship, together with vitamin D, was most important for the formation of teeth, bones, and attaching tissues.

Prinz (1921) studied the origin of salivary calculi. Teeth were imbedded in modeling compound and narrow spaces were left on the buccal and lingual sides beneath the gingival margins to simulate pyorrheal pockets. They were kept at a temperature of 40° and the pockets were filled daily with fresh saliva during 8 to 10 weeks. They presented typical tartar incrustations on their roots identical with the salivary calculi occurring upon human teeth. The same results were obtained when this experiment was repeated, using a synthetic saliva prepared from dried ablumin, carbon dioxide and the following inorganic salts: KCl, K_2SO_4, K_3PO_4, Na_3PO_4, $Ca_3(PO_4)_2$ and $Mg_3(PO_4)_2$.

McIntosh, James and Barlow (1922) found a definite type of bacillus constantly present in carious material. These bacilli produced a high degree of acidity by the fermentation of carbohydrates. The average final pH value of 9 strains was 2.75, which is sufficient to decalcify teeth. Teeth left in contact with pure cultures of this bacillus over prolonged periods showed changes almost identical with those found in caries. The teeth

showed erosion of the enamel with penetration of the dentinal tubules and the formation of liquefaction foci. They proposed to name the bacillus, Bacillus acidophilus odontolyticus. It resembles in its resistance to and formation of acid the Bacillus acidophilus of Moro, but biologically there were points of difference.

Hall (1923) made determinations of the pH changes in the saliva under various conditions. A carbohydrate residue favors fermentation with acid formation and a protein residue favors putrefaction with alkali formation. It seems probable that the microbial changes in the mouth depend primarily on the kind of pabulum as well as on the kind of organisms. He stated that halituous breath may or may not accompany salivary putrefaction.

Diet and Tooth Development in Dogs.—M. Mellanby (1918) studied the formation and calcification of the teeth and jaws in dogs as affected by diet. She interpreted her results to mean that some substance or substances similar in distribution to vitamin A have a potent effect as regards calcification of the teeth. Later work (1923) showed that puppies fed during the period of development of the permanent teeth on diets deficient in A, showed one or more of the following defects of the teeth and jaws: (1) thick and poorly calcified jaw bones and alveolar processes; (2) irregularity in the arrangement of the teeth, as a rule more noticeable in the lower incisors; (3) delay in and retardation of the eruption of the permanent teeth; (4) interference with the calcification of the enamel which is often either deficient in amount or defectively formed; (5) interference with the calcification of the dentine, which may be either small in amount or poorly calcified, containing varying numbers of interglobular spaces; (6) abnormal development of tissue at the gingival margins and the peridental membrane.

The daily diet of the pups consisted of 175 c.c. of separated milk, white bread (70 per cent. wheat) ad libitum, linseed oil 10 c.c., and yeast 10 gm. In addition, one received 5 gm., a second 10 gm., and a third 50 gm. of meat per day. From the seventh to the fifteenth week the dog receiving 10 gm. of meat was also given 10 gm. of butter fat daily. The enamel of most of the teeth was poor, especially in the case of the dog receiving 50 gm. of meat per day. This pup grew the fastest but had the worst teeth. The enamel was best in the teeth of the dog receiving the butter as well as 10 gm. of meat per day.

When the pups were given diets containing a sufficient amount of fat-soluble vitamins, although in other respects the same as above, the jaws, alveolar processes and the teeth were well calcified and the latter were regularly arranged. She states: (1) diminishing the calcium in the food as well as the fat-soluble vitamins resulted in the formation of badly formed teeth; (2) diminishing the calcium in the food, when the fat-soluble vitamin content was high, reduced the amount of calcification, especially that of the dentine of the teeth. The dentine which was laid down was badly formed; (3) increasing the calcium in the food, and diminishing the fat-soluble vitamins, resulted in teeth badly formed although better than under condition 1; (4) increasing the cereal in the diet, when the fat-soluble vitamins were deficient, resulted in worse formed teeth and jaws; (5) cereals differed in their detrimental action on tooth formation. Oatmeal was the worst of those examined, white flour and rice the best.

The addition of 20 per cent. of wheat germ to white flour had a definitely bad effect on tooth formation when fat-soluble vitamins were deficient. When oatmeal was included in a diet deficient in fat-soluble vitamins, the teeth produced were extraordinarily bad. She believes that just as fat-soluble vitamins can overcome to a large extent a deficiency of calcium in the diet, so also can they neutralize the harmful effects of oatmeal. She (1923) further stated that there is a direct relationship between the structure of the teeth and caries, badly formed teeth being more susceptible to disease, whereas those well formed were immune. She (1924) stated that when the diet is only "moderately defective" exposure to radiation has a decidedly beneficial effect on the calcification and structure of the teeth. If the diet produced very defective teeth, improvement by radiation was less, and if the diet was very unbalanced and defective, then the radiations made the teeth even worse. When the radiations were of small value, owing to the pronounced dietetic defects, the latter could be completely counteracted by cod liver oil.

Mellanby, Pattison and Prowd (1924) reported experimental work with children in which they were fed diets comparable to those which Mellanby (1918) had shown in the case of puppies to result in the formation of faulty as well as of well-calcified teeth. The following table gives the daily diets which they fed to the children:

TABLE XLVI

Diet	*A*	*B*	*C* (*Hospital Diet*)
Milk	2 to 1½ pints	¾ to ½ pint	1½ pints
Bread	2 to ½ oz.	5 oz.	Ad lib.
Fat	1 oz. (butter)	1 oz. (margarine except towards end)	1 oz. (margarine except towards end)
Sugar (including cooking)	1½ oz.	3 oz.	1½ oz. and jam, etc.
Oatmeal, groats or gruel		2 to 4 oz.	Occasionally
Rice, tapioca, etc.	½ oz.	½ oz.	½ oz.
Meat (cooked)	1½ oz.	1½ oz.	2½ oz.
Potatoes	2 oz.	4 oz.	4 oz.
Other vegetables	2 oz.	½ oz.	½ oz.
Cocoa	Made with milk	Made with water	Made with milk
Egg	1	Very little	Sometimes
Fruit, etc	¼ orange, or ½ tomato, or swede juice 1 or 2 dr.	As in diet A	As in diet A
Cod liver oil	3 to 6 dr.		2 to 3 dr.

They interpreted the quality of the diet as follows: A, abundant, calcifying vitamin and calcium; a small amount of carbohydrate, not including oatmeal; B, less calcifying vitamin and calcium; much carbohydrate, including oatmeal; C (hospital diet), intermediate amount of vitamin A, calcium and carbohydrate, some oatmeal. It is difficult to understand why Mellanby and co-workers did not feed a diet containing an abundance of the calcifying vitamin (vitamin D) and calcium, and include oatmeal, so that their series would have been complete enough to warrant their deduction that oatmeal is injurious to teeth. Since their diet B contained a very small amount of milk, much oatmeal, and no cod liver oil, one would expect to have poor skeletal growth as well as faulty teeth. We think the data presented by Mellanby and her co-workers do not warrant the conclusion that oatmeal contains a decalcifying factor.

Marshall (1923) examined the teeth of the dogs used for experimental studies by Jones (1924) fed as follows: The basal diet consisted of bread, cooked lean beef, skimmed milk powder, butter fat, and fresh orange juice made into a mush with distilled water; and a salt mixture, the composition of which was based on ash analyses of cow's milk except that the phosphorus was omitted and different proportions of calcium were used. The phosphorus content of the diets was varied by adding dibasic

potassium phosphate to the above salt mixture, and the degree of alkalinity regulated by the addition of HCl. There were several groups of puppies and different groups were fed different amounts of phosphorus.

Jones reported rickets in some of the dogs. The condition was apparently the phosphorus sclerosis described years ago by Dibbelt, Lehnerdt, Stoeltzner and others. Almost without exception the diets which have been employed for the production of rickets in dogs have not been properly constituted for the production of the disease in the rat.

Man and dog are similar in that they both have deciduous and permanent teeth. Nine pups of the same litter were fed a restricted diet from September 25 until they were sacrificed the following May and June. They had had vitamins and sunlight, but apparently not enough for an improperly constituted inorganic supply. The specimens revealed at least two abnormalities in the teeth: a marked delay in dentition, and a relative absence of dentine, although there was a normal amount of enamel. The deciduous teeth were apparently normal in size and shape but were not shed in the usual manner. The permanent set, the incisors, cuspids and molars of the mandible were more generally affected than were the corresponding teeth of the maxilla. An outstanding feature was the retention of the deciduous teeth. Comparable defects were observed in the teeth of other dogs. These results seem to point definitely to a profound effect of faulty diet on the development of the teeth. Marshall (1924) discussed the several hypotheses which have been advanced to explain the etiology of dental caries.

Influence of Diet on Teeth and Bones.—Touverud (1923-24) studied the influence of diet on teeth and bones. He found the normal orthodentin to be largely substituted by osteodentin in the teeth of guinea pigs fed a scorbutic diet. He found the osteodentin incorporating degenerated odontoblasts to reach far down toward the apex of the tooth and there was a very narrow space of orthodentin surrounding it. The pulp tissue became so degenerated that one could scarcely see its normal elements. The process has its beginning with hemorrhages in the upper part of the pulp and extends down toward the apex. The dentin seemed to be more affected than the enamel. He found a great reduction of total ash and calcium oxide, but an increase in magnesium of the teeth of scorbutic, as compared with normal animals.

He also studied the teeth of rats on faulty diets. The diet

consisted of whole wheat 92, butter fat 5, a complete salt mixture, but without calcium. The total ash, calcium and phosphorus in the bones of treated adult rats were very much below those of normal rats; the magnesium content was distinctly increased. The chemical changes in the teeth were similar to those in the bones. He concludes that the resistance of the teeth against decay must lie in their structure and chemical composition. Changes in these two factors may be the first step in the process of decay. The changes may be quite small, but sufficient to render the tooth susceptible for a third factor, which may be a local one, but which may be the final step in the process of decay.

Howe (1926) stated that in man the magnesium content of carious teeth is practically twice that of sound teeth; he confirms the work of Touverud (1923-24) that the magnesium content of the teeth of scorbutic animals is higher than in normal animals. Howe regards dental disorders as fundamentally due to irregular mineral metabolism.

Mellanby (1928) found that the provision of vitamin D with a diet which without it was defective for tooth development, caused the formation of smooth, white, glistening, regularly arranged teeth in dogs. On microscopic examination these showed evenness of calcification in both enamel and dentine; dogs on the same diet without the vitamin had rough, pigmented teeth which were irregularly arranged in the jaws and which showed marked defects in calcification of enamel and dentine.

Nutritional Factors in Preventive Dentistry.—From what has been said in this and other chapters it will be evident that certain people in the past, and certain people of the present time, have escaped the ravages of dental disease, which is now such a heavy burden in America and in certain European countries. Our present incomplete knowledge indicates that diseased teeth result from: (1) eating foods which do not demand chewing and consequent exercise for the teeth; (2) adherence of pasty foods to the surface of the teeth with resulting fermentation and attendant acid formation which etches the enamel; (3) unhygienic condition of the mouth; (4) probably also, abnormal composition of the saliva which, when normal, should protect the teeth.

The development of sound teeth, capable of resisting the destructive agencies, is essentially a dietary problem. The small jaws and crowding of the teeth so frequently seen in children is

almost certainly the result of faulty skeletal development, and will be intensified by dietary errors which favor the development of rickets. Mal-position favors food packs and the maintenance of foci of fermentation between the teeth. The evidence presented strongly indicates that failure of normal tooth formation —by which we mean the proper spacing of sound teeth—during the developmental period, is the basic cause of a series of events which termintes in dental caries.

It seems probable that the condition of the mouth is made unfavorable to the maintenance of health of the teeth by reason of people eating regularly to the point of surfeit as is generally the case where food is plentiful and economic conditions favor good living. Overeating results, among other things, in digestive disturbances, an incident of which is the regurgitation during sleep of small amounts of stomach contents. Although the latter are in great measure immediately reswallowed, enough material remains to support the profuse "fur" frequently seen on the tongue. The products of the metabolism of such excessive growth of micro-organisms can reasonably be considered injurious to the teeth. It would be instructive to observe the effects of comparative abstemiousness in a group of persons with furred tongues and pronounced tendency to caries.

CHAPTER XXIX

THE RELATION OF THE DIET TO RESISTANCE TO DISEASE

Dietary Deficiencies and Infection.—The conviction has grown in recent years that several conditions, such as scurvy, beri-beri, xerophthalmia and pellagra, arise not primarily from the presence of an invading organism but largely as a result of lack of certain indispensable constituents of the diet. This knowledge has stimulated inquiry into the concomitant change in resistance to bacterial invasion in such pathological states. Few would now reject the array of experimentally established facts and deny the relation of these diseases to lack of some essential substance in the diet, or question that there is a high degree of specificity in the effects of the missing constituents which induce the development of these syndromes.

Importance of Animal Experimentation to Human Welfare.—The sciences of medicine and surgery are built upon experience gained through animal experimentation. Most of our knowledge of the rôle which bacteria play in the production of disease has been gained through studies on the lower animals. Protective serums could never have been discovered except by such procedure.

The latest branch of medicine to develop is that of preventive medicine. This new field owes much to animal experimentation. Natural vitality enables the tissues to resist the entrance of certain bacteria. An optimum condition of the tissues constitutes an effective defense for the body against certain kinds of diseases. Our knowledge of the profound effect which the diet may exert on the vigor of the body is due to observations of the effects of specially planned experiments on small animals. The study of the science of nutrition as a branch of preventive medicine has not been sufficiently appreciated in the past.

Value of Scientific Nutrition in Preventive Medicine.—The researches in the field of nutrition have a greater value in preventive medicine in relation to raising the vitality of mankind,

with all that this implies, than they have in the prevention of the deficiency diseases. This fact has never been sufficiently appreciated. In the aggregate, by far the most important effect of faulty nutrition in man is the result of errors of one kind or another which are not sufficiently grave to cause prompt and spectacular failure. It is the gradual operation of more or less constant, but unperceived causes, rather than of great exposures of an accidental nature which in most cases are responsible for undermining the health of the individual. Of these causes it now seems certain that the consumption of an improperly constituted diet is one of the most important. It is one of the causes of inferiority in physical development, instability of the nervous system, lack of recuperative power and endurance and consequent cumulative fatigue, and lack of resistance to infections such as tuberculosis and other diseases where specific immunity is not easily developed by the body. In addition to these, the rate of development of senile characteristics and consequently the length of the span of life are greatly influenced by the type of diet to which one adheres.

Physiological Effects Due to Vitamin-free Diets.—Baldwin and associates (1924) measured the blood pressure in the abdominal aorta of the rat and found the average normal blood pressure to be 84 mm. of mercury. Rats on a vitamin A-free diet showed an average sub-normal pressure of 75 mm. Vitamin B deficient animals showed a marked sub-normal pressure, the average value being 34 mm.

Magne and Simonnet (1922) found that pigeons suffering from lack of vitamin B show a respiratory quotient below the normal value, and Baldwin and co-workers (1928) demonstrated the same phenomenon with chicks on a rachitic diet. The quotient dropped from 1.0 to 0.7. Nelson and associates (1925) studied the effect of vitami deficiency on muscle fatigue. The fatigue curves of animals on diets lacking vitamin B showed a progressive decrease in amplitude and endurance with increasing length of time on the diet. There was a progressive loss of tonicity and a characteristic irregularity of contracture. Similar fatigue curves were obtained from rats showing symptoms of xerophthalmia.

Sprue and Lowered Resistance.—Ashford and Hernandez (1926) assert their belief that sprue is the result of infection with Monilia psilosis, susceptibility being conditioned upon chronic digestive incompetence, and that thrush in infants is due to in-

fection with Monilia albicans, due to lowered resistance of the marasmic infant.

Fat Absorption.—Mottram, Cramer and Drew (1922) made a histochemical study of fat absorption from the intestine. The functional activity of the intestinal epithelium as regards absorption is profoundly affected by the presence in the food of vitamins A and B, particularly the latter. In the absence of vitamins they found no delay in the passage of food in such animals.

Natural Vigor and Susceptibility to Tuberculosis.—There are many who doubt whether the adherence to a faulty diet has anything to do with susceptibility to tuberculosis, but there is much reason to believe that it has. Experience has shown that there is no more effective therapeutic measure than proper feeding to raise the recuperative power of the patient. If such treatment affords greater prospect for throwing off the disease once it is established, it would be decidedly paradoxical to maintain that highly satisfactory nutrition could play no important rôle in protecting the individual against the establishment of the infection. Appleton has pointed out that the men of Labrador and Newfoundland suffer greatly from tuberculosis, although they spend at least half of the year out-of-doors in an invigorating climate. She states that owing to the strong winds which blow almost constantly during the winter, the houses are ventilated so thoroughly as to make garments wave which are hanging in the rooms for drying. She suggests that the faulty diet is the predisposing cause rather than unhygienic housing.

Effect of Diet on Experimental Tuberculosis.—Gloyne and Page (1921) observed that rats fed a diet deficient in vitamin A and inoculated with tubercle bacilli died earlier than uninoculated animals on the same diet. The deaths were due to non-tuberculous pneumonia. Tubercle bacilli were present at death in the spleen and blood of inoculated rats irrespective of the diets received.

Lange and Simmonds (1923) noted no significant difference in the reaction to infection of rats infected with bovine tubercle bacilli by subcutaneous inoculation on diets of varying protein content either in the general condition, weight curves, or in the gross or microscopic autopsy findings. Rats on a diet deficient in salts but otherwise adequate, showed a more diffuse and extensive local reaction at the site of inoculation and a slower dissemination and a slower elimination of the lesions. Lange (1925) has continued the study of the effect of deficient diets on

the susceptibility of the rat to experimental bovine tuberculosis. The course was essentially the same in the control and test diet groups with the single exception of the group on a diet high in fat but deficient in vitamins, which showed an earlier complete elimination of the tubercle bacilli. She also found that a deficiency in the protein or salt factors affected the susceptibility of the rat to bovine tuberculosis more than any other factors.

Rickets and Tuberculosis.—A most interesting series of experiments have been reported by Grant and co-workers (1927). They kept young rats under conditions and on a diet suitable for the production of rickets. When these animals were injected with tubercle bacilli the disease could be demonstrated not only by microscopic study of the tissues but also by transmitting it to guinea pigs. The series given the defective ration alone developed rickets but no tuberculosis, while doses of the organisms many times larger than those used in the rachitic group failed to induce tuberculous lesions in a number of rats given an adequate ration. It is well known that the rat is highly resistant to tuberculosis when the organisms are given subcutaneously. Similar results were obtained when the experimental ration lacked only vitamin D for several generations. Rickets was produced, and with it a susceptibility to tuberculosis. It appears that a marked lowering of resistance to this type of infection was the direct result of dietary deficiency.

Relation of Vitamins A and B to Lymphoid Tissues.—Cramer, Drew and Mottram (1921) observed that absence of vitamin B from the diet of mice and of rats led to atrophy of the lymphoid tissue throughout the body and also to lymphopenia in the circulating blood. The number of polymorphonuclear leukocytes was not affected. The absence of A did not lead to atrophy of the lymphoid tissue and there was no lymphopenia. Absence of vitamin B led to characteristic nutritional disturbances, such as loss of weight, emaciation, and subnormal temperature, which collectively may be designated "marasmus." No such marasmic condition resulted from the lack of A. This condition can be produced by other processes which destroy lymphocytes, such as X-ray exposure, indicating that the lymphocytes play an important rôle in nutrition. They conclude that vitamin B is necessary for the normal functioning of the lymphoid tissue.

Vitamin A and Blood Platelets.—Cramer, Drew and Mottram (1922, 1923) reported that a diet deficient in A produced in the rat a progressive decrease in the number of blood plate-

lets. This thrombopenia was the only constant lesion for vitamin A deficiency and was characteristic of this deficiency, just as a lymphopenia was characteristic of a deficiency of vitamin B. Thrombopenia may be present in rats on a diet free from A before they show any signs of ill health. When a profound thrombopenia had been established, addition of A to the diet produced a rapid increase of platelets to the normal number, provided the animals had not suffered too long and too severely from the vitamin deficiency. Exposure to radium produced a lymphopenia and also a thrombopenia if the dose was sufficiently large, but the animals recovered rapidly if the radium treatment was discontinued and the dose had not been too large. When the number of platelets had been reduced below a certain critical level (approximately three hundred thousand in the rat) by exposure to radium or withholding vitamin A, resistance to infection was greatly decreased, and infective conditions developed spontaneously and often gave rise to secondary anemia. These infective conditions cleared up when the number of platelets increased. They believe that the blood platelets have an important function in the mechanism of resistance to bacterial infection, and that alterations in local or general resistance to infection are associated with local or general changes in the distribution of the platelets.

Bedson and Zilva (1923) reported that rats fed diets deficient in vitamin A show a decrease in the number of platelets, but believed the decrease too small to be significant. No recent work has been done on this problem.

Light and Formation of Platelets.—Cramer (1923) advanced the view that vitamins have a drug-like action on the alimentary tract, and maintain integrity of the apparatus for digestion and absorption. He believes vitamin B has a specific stimulating influence on the lymphoid tissue, and that vitamin A has a like influence on the intestinal mucous membrane as well as on the formation of platelets. He (1923) found light to be a stimulus to the formation of blood platelets but not an essential condition. Its absence could be counteracted by an abundance of vitamin A.

During the past few years much work has been done in an endeavor to control the growth of tumors by diet. Little if anything has been accomplished, but some of the attempts are here recorded.

Vitamin A Deficiency and Tumor Growth.—Wyard (1922)

reports that in the treatment of malignant disease by a diet free from vitamin A, in no case was any effect of the diet noted on the growth and evolution of the tumor, which invariably progressed along the usual lines. Copeman (1923) reports an investigation of the ultimate effect on the adult of a diet deficient in vitamin A; he believes the adult requirements to be extremely small. He placed cancerous patients on a diet from which foods of animal origin containing A were excluded, in the hope of obtaining a differential effect on the growing tumor; he believes this treatment afforded in some cases increased expectation of life and reduced the pain sufficiently to obviate the need of anodynes.

Relation of Avitaminosis to Tumor Growth.—Cramer (1923) stated that the growth of cancer cells cannot be arrested by withholding vitamins from the host. With tryptophane deficiency the tumor grows, although the growth of the animal is arrested and some tissues even atrophy. Dietary restrictions of a degree to impair the health of the host do not arrest the growth of the tumor. McCarrison (1923) induced avian polyneuritis in pigeons and then cured them by a satisfactory diet; 6 to 12 weeks afterwards 7 of the 12 developed epithelioma contagiosum, which was attributed to their poor nutrition during the initial experimental stage. He says: "the state of faulty nutrition favorable to the operation of the invisible virus of this malady is brought about by deficiency of certain substances in the diet, among which vitamin B is one."

Vitamin A and Cell Growth.—Jorstad (1925) introduced drops of coal tar into the subcutaneous tissue of animals. These injections attracted fibroblasts, and endothelial and other cells. These cells suffered degenerative changes through the action of the tar, and the animals suffered cachectic-like changes and death from large doses of it. The action of the tar is limited to a short period, after which it becomes inert. The cells which have been drawn to it and which have not completely degenerated them slowly recover. Vitamin A fed in more than ample quantities to these animals protects the cells against the toxic action of the tar and stimulates and prolongs their secondary growth.

Vitamin-free Diets and Tumor Growth.—Ludwig (1926) states that rats and mice kept on a vitamin-free diet as long as possible and then inoculated with malignant tumors either fail to develop these tumors or outlive the control animals, all of

which develop tumors. The animals were given vitamin-containing foods when it became necessary to keep them alive.

"Archusia" and "Ergusia."—Burrough and Jorstad (1926) state that body cells can grow only when acted on by a certain concentration of a substance or substances formed by them or by other cells called "archusia," which they believe to be identical with vitamin B. Cancer, they believe, is normal growth of which body cells are capable when released from the effects of active circulation and crowded into a sufficiently stagnant environment. Processes of differentiation, function, formation of matrix of bone, extracellular formation in general, and the storage of fat in cells are dependent on the continuous liberation of a soap-like substance—ergusia, which they believe identical with vitamin A. Continued activity soon exhausts these cells unless vitamin A is supplied from endogenous sources. They regard vitamin A as essential for the growth, proper structure, and function of higher metazoon life, but believe it is not needed by yeast, bacteria, and many lower unicellular forms.

Carcinoma in Rats as Influenced by Diet.—Sugiura and Benedict (1926) found transplants of carcinoma in rats fed a complete basic diet but limited in quantity to one-third the normal food requirement survived less frequently and grew much more slowly than those of full fed controls. Under-feeding, after cancerous grafts had well-established themselves in the hosts, had no marked inhibitory effect on the subsequent rate of tumor growth. Prolonged post-operative insufficient feeding had a distinct influence in reducing the frequency of recurrences of spontaneous tumors in mice. The average post-operative longevity in underfed mice was 142 days as against 96 for the full fed controls.

Studies on Relation of Diet to Disease.—Smith (1913-14) found that epidemics of pneumonia due to the pneumococcus and B. bronchisepticus were very common in guinea pigs during the winter months when the diet was deficient in green food, while in the summer, when an abundance of green food was supplied, no cases of pneumonia developed.

Setti (1922) found pigeons fed polished rice to be less resistant to attenuated infections than normal birds, and the latter developed a higher immunity when vaccines were employed. Stolte (1922) found that the ability of the organism to resist infection is reduced by a vitamin-deficient diet. He believes that vitamin-rich foods enhance resistance. Bickel (1922) concluded from a

series of experiments that a vitamin-rich diet increases resistance and that diet is a factor in immunity.

Findlay (1923) observed that diets lacking in vitamin B produced in pigeons and rats congestion and hemorrhage in the hemopoietic tissues and in the bone marrow, followed in the more chronic cases by a gelatinous degeneration. Pigeons fed a diet lacking in B were much more susceptible to infection with the pneumococcus and the meningococcus—organisms to which they are naturally quite immune—and to infection with B. coli, and with B. enteritidis (Gaertner). The reduction of natural immunity he associated with the fall of body temperature produced by lack of vitamin B. It was marked only when the cloacal temperature was lowered to 40° or below. Lowering of the body temperature appeared to decrease the resistance by (a) facilitating the growth of the invading organisms, (b) reducing the leukocytic response to the infection, (c) reducing the bacteriological power of the leucocytic exudate. He (1923) discussed the relation of vitamin C to infection. Guinea pigs were infected with the pneumococcus, Staphylococcus aureus, Streptococcus haemolyticus, and B. coli. Animals suffering from a lack of vitamin C succumbed to a smaller infecting dose of bacteria than did the normal animals. Since in chronic scurvy there appears to be degeneration in the bone marrow, this is suggested as one of the factors responsible for the reduced resistance.

Mouriquand, Rochaix and Michel (1923) reported that guinea pigs on a scorbutic diet do not assume the hemorrhagic character any more than do animals on a satisfactory diet when suffering from diphtherial infection. When infected with Bacillus pyocyaneus and anthrax bacilli, the development of lesions seemed to be more serious and more extensive in the control than in the undernourished animals. Coulaud (1924) says the progress of experimental tuberculosis in guinea pigs is surprisingly regular, and is greatly hastened by depriving the animals of vitamin C. Webster and Pritchett (1924) reported that the white mice from the Rockefeller Institute breeding room fed on the McCollum complete diet consisting of whole wheat 67.5, casein 15, milk powder 10, NaCl 1.0, $CaCO_3$ 1.5, and butter fat 5.0 per cent., are more resistant to mouse typhoid infection, mercuric chloride intoxication, and botulinus toxin, than are similar mice fed on bread and pasteurized milk supplemented by oatmeal and buckwheat mixture, and dog biscuit.

Smith, McClosky and Hendrick (1926) observed increased susceptibility to pilocarpin and ergotoxin in rats with vitamin B deficiency. Rats on A-deficient diets showed much lower resistance to ergotoxin and to morphine. Definite though slight increase in susceptibility was also noted to histamine and to strychnine, atropine, cocaine, and apocodein.

Parrino and Scarpulla (1927) found the phagocytic power of guinea pig and pigeon leukocytes is reduced by avitaminosis and may be restored by vitamin feeding.

Immunological Effects of Lack of Vitamins.—Hektoen (1914) reported that rats stunted because of low protein content of the diet did not show any disturbance in the production of antibodies. Wertheimer and Wolff (1921) studied the incidence and duration of infection, and mortality rates in several groups of children. Striking differences were observed, which were all in favor of the adequately-nourished children. These investigators believe that since antibody formation is a function of the cell, any condition, such as marked undernourishment, which tends to impair the activity will be reflected in a decreased resistance of the body to infection.

Zilva (1919) studied the influence of poor nutrition on the production of agglutinins, complement and amboceptor. Guinea pigs were fed an unrestricted mixed diet, a quantitatively restricted mixed diet, and a scorbutic diet. There was no difference in the amboceptor and agglutinating titers, nor in the complement activity of the blood. Rats were fed on diets low in calcium, low in iron, low in potassium, low in sodium, low in chlorine and low in phosphorus, low in protein, and also diets deficient in vitamins A and B. Although several of the deficiencies became manifest by restricted growth and poor condition, no reduction in the titers of the agglutinins or the amboceptor could be found in any of the groups except those receiving the low phosphorus diet.

Findlay and MacKenzie (1922) found that rats on diets deficient in vitamins A and B showed no increase in the opsonic activity of the serum. The phagocytic activity of the polymorphonuclear leukocytes of the guinea pigs with chronic scurvy was not increased. Hamburger and Goldschmidt (1922) found the serum of scorbutic children and animals to contain the same amount of normal amboceptor and complement as the healthy controls. Hilgers (1922) found the complement titer in undernourished guinea pigs to be reduced.

Werkman (1923) made a study of the importance of vitamins A and B in immunology. He demonstrated that rats and rabbits when given vitamin A-deficient diets develop marked increase in susceptibility to anthrax and pneumonia. Rats and pigeons lacking vitamin B were likewise more susceptible to these infections. The relation was therefore not specific for either vitamin. With B. typhosus and a staphylococcus, rats lacking vitamins A and B showed a slightly decreased but consistent phagocytic index. Werkman concluded that the depression of the activity of the phagocytes was not due to failure in production of opsonins but was caused by some depressive agent acting on the mechanism of phagocytosis. He suggested a lowered body temperature, which is characteristic in certain avitaminoses, as the cause. Werkman and associates studied the cause of increased susceptibility of scorbutic guinea pigs. There was marked decrease in their resistance to pneumonia and anthrax, but they could not refer this to depression of phagocytic activity, which was the case with rats. Guinea pigs lacking C showed the same ability as healthy animals in the production of specific agglutinins for typhoid bacilli. They believe a lowered body temperature is of profound importance in facilitating infection. They (1925) also studied the relation between avitaminosis and susceptibility of the rat to diphtheria toxin. Rats deprived of either A or B succumbed to the injection of smaller doses of diphtheria toxin than normal animals given a complete diet. The deficient animals were still able to produce antitoxin. It was concluded from this experiment that "the drop in resistance of avitaminic rats to diphtheria toxin is the superimposing of deleterious effects rather than a break in the immunity mechanisms."

Koch and Smith (1924) reported in scurvy a definite increase in the complement titer over that of the fore period. During convalescence the titer remained somewhat higher than in the fore period. Starvation resulted in a lowering of the titer.

Sartori (1925) kept guinea pigs on a vitamin-deficient diet and observed that they became hypersusceptible sooner than normally-fed controls to the sensitizing action of injected protein. Avitaminosis had no influence on a preëxisting state of sensitization.

Relation of Vitamin A Deficiency to Infection.—One of the most critical discussions of the relation of deficiency of vitamin A to infection has been reported by Sherman and Burtis (1928).

They have studied the long-delayed effects of a deficiency of vitamin A "no greater than may readily occur within the range of ordinary normal or adequate nutrition." Two large groups of rats were taken at four weeks of age: the first group had been reared prior to this time on an adequate ration with a safe margin of A, and the second group on a similar complete food but with less vitamin A. All the animals were then depleted of A by being given for one month food free from this factor. Both groups were then fed for 8 weeks a diet containing a limited concentration of A. At the end of the experiment, autopsies showed that in the first group there were infections in 25 per cent., while in the second group, 75 per cent. were infected. The only difference in the two groups was the dietary treatment of the mother and young up to the time of weaning, one group having plenty of A, and the other a somewhat limited supply. The results support the view that a relative deficiency of this principle in infancy exerts a lasting deleterious effect.

One year in the age of a rat is the equivalent of about 30 years in man. On this basis the authors remark that one may find here an index of incidence of infection to be expected among children of about ten to twelve years of age resulting from differences in the way they were fed before they were three years old. The often-expressed consolation that "the child will outgrow" this or that abnormality is an unwarranted attitude. It is well to call attention in the light of such data that complacency without an intelligent effort to discover the cause of malnutrition in childhood may effect far-reaching and unfortunate consequences.

Macy, Outhouse, Long and Graham (1927) have suggested that the failure of many young rats to grow after a period of stunting from vitamin A deficiency, when an abundance of the latter is added to the diet is due to the prevalence of infection resulting from vitamin A starvation. They found the presence of keratinized epithelial cells in the vaginal smears from virgin rats an early and reliable sign of vitamin A deficiency. Goldblatt and Benischek (1927) have shown that the same type of epithelial keratinization was induced in rats receiving vitamin D but no A as when D was not supplied, showing that the metaplasia is specifically the result of A starvation.

Hume and Smith (1928) have stressed the importance of adding vitamin A from the beginning of the experiment and of extending the observations over a long period in the biological

assay of foods because of the prevalence of infections in the curative type of biological assay for this vitamin.

Fujimaki (1928) reports the experimental production of carcinoma of the stomach in rats by prolonged underfeeding of vitamin A. Hyperkeratosis of the gastric mucosa under these conditions has been observed by others, but no one else has reported the development of malignancy.

Osborne and Mendel (1917) described the formation of urinary calculi in rats fed diets deficient in vitamin A. This has been confirmed by Fujimaki (1926); Van Leersum (1928); and by McCarrison (1927).

Green and Mellanby (1928) also report an investigation on the effect of vitamin A deficiency on susceptibility of rats to disease. Ninety-three were given the A-deficient diet, and fifty were maintained as controls, receiving cod liver oil, butter fat or dried cabbage as a source of vitamin A. The ninety-three rats deprived of A grew fairly well for a time, then refused to eat, lost weight and died. Postmortem examinations showed that all but two had infections in one or more parts of the body. Abscesses at the base of the tongue, involving the salivary glands and lymphatic glands of the neck, were found in 72 per cent. of the animals. Infections of the kidney and bladder occurred in 44 per cent. The alimentary canal was the seat of infection in 21 per cent. Pus was found in the middle ear or nasal sinuses in 20 per cent. The incidence in the lungs (septic-bronchial pneumonia) was 9 per cent. The reproductive organs were frequently acutely inflamed or suppurating.

None of the fifty control rats had any gross infective lesions. The only pathological conditions found were three cases of hepatic cyst, due to parasites. The authors conclude that vitamin A is more directly related to resistance in infection than any food factor of which we are aware.

Their investigation also included a study of the effect of vitamin A with provision of vitamin D. They state "It often appeared that the addition of vitamin D in the absence of vitamin A hastened the onset of the infective condition."

The importance of vitamin D has attracted great attention recently and it has even been suggested that preparations of vitamin D can safely be substituted for cod liver oil in medical treatment. The work above described shows that this teaching is erroneous, and that, although vitamin D controls, probably absolutely, the calcification of bones and teeth, compared with

vitamin A it has no direct power to promote resistance to infection in the same way as vitamin A. If a substitute for cod liver oil is given it should be at least as powerful as this oil in its content of both vitamins A and D.

The experimental work just discussed confirms the views of Cramer (1922). He stated his belief at that time that if children are fed too little of the vitamin-rich foods during infancy there is a lasting weakness which manifests itself if the child is exposed to a strain. He believes there is a social aspect of the vitamin problem which has not been recognized and that this is more important than the actual production of severe vitamin deficiency. The physical make-up of a community, he stated, is determined largely by the ease and regularity with which an abundant supply of vitamin-containing foods is available for the pregnant and nursing mother. It is of the greatest importance to appreciate the fact that there may occur by reason of faulty diet serious damage to the young without the development of symptoms which definitely mark the sufferer from a deficiency disease. Many malnourished children find their way into the school clinics. We do not desire to minimize the importance of after-effects of infectious diseases as a factor in swelling the number in this group of children. In a very large number of cases, however, there can no longer be any reasonable doubt that the most fundamental problem is that of securing for these children a wisely planned diet which will raise their vitality to a higher plane.

Importance of Water in Health and Disease.—The maintenance of health at a high level by such important agencies as good nutrition and adequate rest seems to be intimately associated with our ability to resist various diseases. Many persons who consider they have good health would doubtless enjoy a greater sense of well-being if they increased their consumption of fluids. With few exceptions water, like air, is so constantly available that its potential benefits are in great measure not appreciated. Undoubtedly most of us do not drink enough water or other fluids or partake of foods which are high in their water content to meet the water requirements of the body; the healthy man requires about 2500 c.c. (about 2.5 quarts) per 24 hours. The importance of an adequate amount of water is obvious when we reflect that about 70 per cent. of the entire body weight is water. The daily consumption of a quart of milk—that unique and important food to which we have frequently made reference

—would tend to insure an adequate water intake. Of the several rôles which water plays in the body, mention may be made of that of food solvent, as a vehicle for the removal of waste products, and as a factor in the regulation of body temperature. Water may be taken with impunity at meals, but never to wash down food.

Only comparatively recently physicians have found that the prescription of large amounts of fluids is one of their most valuable aids in treating certain diseases.

It is Impossible to State Minimum Requirement for One Factor Unless All Dietary Factors Evaluated.—Some of the confusion which is evident from studies recorded in this chapter may be accounted for through failure to plan the experimental diets with sufficient care. It is impossible to state the amount of any food factor which constitutes the minimum or the optimum, or an excess, without taking into consideration the biological values of each of the other factors in the diet. The minimum amount of any dietary factor of constant quality which will just serve to maintain apparently normal well-being will not suffice when another factor is modified so as to be unsatisfactory; but after such a modification of the diet, increasing either of the two factors under consideration will in some measure protect the body from harm. Hence there is a principle of relativity which is of fundamental importance in estimating the value of a diet as a whole. The type of experiments here described illustrates how animals may be brought into a "twilight zone" of nutritional instability and yet present no external evidence that they are not in the optimum condition.

CHAPTER XXX

NEW VIEWPOINTS IN HUMAN NUTRITION

The period of growth of the young in the human species is extraordinarily long as compared with that of any of the mammals among the lower animals. This long period of youth is a factor of safety, since the opportunity for recovery from faulty nutrition at any time during early life is relatively great, because of the long-time interval during which growth is possible.

Our criteria for optimum well-being in animals are: uninterrupted growth to the full adult size; high fertility; low infant mortality; perfection of the bones and teeth; ability to resist infection; and the capacity to reach advanced age before senile characteristics appear. With these criteria as a basis of judgment regarding quality of foods, we are justified in the generalization, on the basis of numerous experimental results from many sources, that for such animals as swine and rats, it is impossible to obtain optimum nutrition on any diet, the selection of which is restricted to any or all of the following groups of food-stuffs: cereal grains, e.g., wheat, corn, rice, etc.; the legume seeds, such as peas and beans; tubers and roots, such as the potato, sweet potato, radish, turnip, beet, and carrot; and muscle meats, such as ham or beefsteak. With any diet, restricted to these sources, in which highly refined cereal products, sugar, molasses, or other nearly pure carbohydrates, find a prominent place, the results in animal experiments will be less satisfactory than where the manufactured foods are replaced by their equivalent in whole cereals, and other vegetables of the types enumerated.

Milk and leaves of plants occupy unique positions among available food-stuffs, in that they are so constituted as to correct, when suitable amounts are included in the diet, the defects of cereals, tubers, roots, and meats. For this reason it was suggested eleven years ago that they be distinguished by the term "protective foods."

In general, there is a marked tendency for the diet of the average American of to-day to be deficient in the element cal-

cium, somewhat over-rich in phosphorus in proportion to the calcium it contains, deficient in vitamin A, and in some cases C and G, as well as the anti-rachitic principle D. Studies with animals have established the remarkable value of milk and leaves as supplementary foods.

With such information available, in 1919 we sought an opportunity to test on human beings to what extent the principles established in animal experiments apply to the nutrition of children.* We sought a group of children who, because of poverty or lack of knowledge on the part of those responsible for their care, were being fed a diet which we suspected to be inadequate for the production of growth and well-being. We discovered an institution in which there were 236 negro children ranging in age from early infancy to twelve years, whose environment and food supply left little to be desired in relation to our objective. They were housed in a building of good construction. It was well ventilated, had good toilet facilities, and had sun porches where the children were instructed in bad weather. There were about 10 acres of ground, partly woodland, where they played out of doors in pleasant weather. The persons in charge of the institution had the best possible attitude toward the welfare of the children, but the financial resources were meager. Severe malnutrition existed in most of them; it appeared that the cause was probably inadequacy of their diet. An opportunity was offered to improve the quality of the diet in order to observe the extent to which the children would respond in growth and well-being.

On ethical grounds it is not permissible to subject children to diets whose quality is suspected of being unsatisfactory, but it is commendable to improve the dietary of children who are, for reasons of poverty or lack of knowledge, being subjected to conditions inimical to their development.

Eighty-four children between the ages of four and ten years were selected for the study. The only basis of rejection was obvious syphilis. A considerable number were believed to be tubercular; many showed rachitic deformities. Since it was of the utmost importance to interfere with the daily routine of the institution as little as possible, and because of inadequate facilities in the kitchen, it was deemed inadvisable to attempt additional cookery in the demonstration. These 84 children were

* The institutional study reported here was made by the senior author in collaboration with Dr. Helen T. Parsons and Miss Ethel Kalmbach.

weighed, measured, and photographed without their clothing. Their ages were in practically all cases accurately known. Examination confirmed the conclusion that malnutrition of severe grade was widespread among them.

A study of the dietary of the institution over a few weeks revealed that it consisted essentially of a soup made of a fairly wide variety of food-stuffs, together with all the bread which the children cared to eat. Cereal was frequently served for breakfast. In Tables XLV, XLVI, and XLVII the food supply over intervals of one week is given in detail. This is fairly typical of the period covered by the study. All the components of the diet except the bread and some cereal and fruit were incorporated in the soup. The soup was palatable and attractive, and was believed to possess a high food value. From the standard of calories and proteins it left little to be desired. During the demonstration 4 to 5 per cent. of the total calories were supplied as meat, which was included in the soup, and added to its attractiveness.

The diet of these children during the period when growth should be proceeding at a rapid rate consisted essentially of cereals, tubers, fleshy roots and muscle meats, and a considerable portion of the food was refined wheat flour in the form of white bread. Since the diet of bread and soup contained no fresh raw food, the question arose why scurvy had not appeared. They showed no scorbutic tendencies, and the attending physician assured us that scurvy had not been a problem. The cause of their freedom from scurvy was soon discovered. Certain fruit vendors in near-by markets regularly donated lemons which were likely to spoil before the next market day. It had been the custom to give each child sliced lemon. This one raw fruit was greatly appreciated by the children. This was the source of vitamin C.

The simplest procedure for improving the quality of this diet without involving additional cookery appeared to be the addition of milk. One group of 42 children received 1 quart of whole milk each per day during the demonstration period. This was prepared from Merrell-Soule's whole milk powder (Klim), by dissolving the proper amounts of whole milk powder in cold water and agitating in a mixer. Only in very cold weather was the water warmed. The other group, designed the check group, was continued on the institutional diet. We were thus in a position to compare the children on the two dietaries, since every effort was made in dividing the 84 children into groups of 42 each

TABLE XLVII

(a) Milk Group

[Diet for two weeks; 40 children]

Food	*Weight*		*Calories*
	Lbs.	*Oz.*	
Hominy	4	12	7,638.0
Rice		10	944.0
Barley	21	0	33,810.0
Flour	1	12	2,805.4
Cornstarch	2	0	3,264.0
Corn meal	6	4	10,081.2
Green corn	1	1	487.7
Oatmeal	35	0	63,105.0
Bread	245	9	288,290.6
Dried beans	5	2	8,015.4
Salt mackerel	3	6	4,698.0
Beef	49	5	34,962.5
Ham	5	2	6,565.2
Pork		5	478.0
Potatoes	80	3	30,310.8
String beans	11	7	2,161.6
Carrots	5	1	1,037.8
Onions	4	6½	969.7
Turnips	1	15	347.0
Beets	4	7	927.7
Cabbage	3	13	544.7
Squash	2	1	443.0
Kohl-rabi	1	5	190.3
Preserves	12	0	19,020.0
Apples	5	0	1,070.0
Bananas	3	12	1,675.8
Condensed Milk		4⅔	448.0
Fat	2	0	6,820.0
Sugar		9⅛	1,029.4
Whole milk (dried)	1,190	0	.....
Total calories from institutional diet	.....		532,191.2
Calories from institutional diet per day per child	.....		950.30
Milk calories per child per day	.....		580.24
Calories per child per day	.....		1,530.54

TABLE XLVII—*Continued*

(b) Institutional Diet

[Diet for two weeks; 92 children]

Food	*Weight*		*Calories*
	Lbs.	*Oz.*	
Barley	8	12	14,087.2
Green corn	5	9	2,553.3
Oatmeal	70	0	126,210.0
Bread	1,165	0	1,367,710.0
Dried beans	2	13	4,398.1
Beef	56	11	40,191.3
Potatoes	1	11	637.6
String beans	19	9	3,697.2
Beets	8	0	1,672.0
Carrots	4	5	884.0
Onions	3	3	701.4
Turnips	1	3	212.6
Cabbage	4	9	652.1
Tomatoes	5	9	573.5
Eggplant	2	6	308.6
Squash	2	10	564.0
Kohl-rabi	2	8	362.48
Cheese	1	14	3,738.4
Condensed milk		9⅓	896.0
Preserves	24	0	38,040.0
Apples	34	8	7,383.2
Sugar		21	2,316.3
Total	.....		1,617,789.28
Calories per day per child	.....		1,256.04

TABLE XLVIII

(a) Milk Group

[Diet for two weeks; 40 children]

Food	*Weight*		*Calories*
	Lbs.	*Oz.*	
Hominy	6	8	10,452.5
Rice	9	5	14,816.0
Barley	17	7	28,074.2
Flour	4	0	6,412.0
Noodles	1	8	2,497.0
Corn meal	4	4	6,855.2
Corn	1	11	774.7
Oatmeal	34	4	61,752.4
Bread	242	2	284,254.8
Beef	69	14	49,541.2
Wieners	1	8	1,701.2
Potatoes	50	4	18,994.4
String beans	10	3	1,925.4
Green peas	1	3	538.9
Carrots	3	7	704.6
Onions	5	6	1,182.8
Turnips	2	8	447.6
Cabbage	3	15	562.5
Celery	2	15	246.0
Kohl-rabi	1	8	217.48
Eggplant	3	10	471.0
Preserves	12	0	19,020.0
Condensed milk		11⅓	1,088.0
Whole milk (dried)	140	0	.....
Fat	2	3	7,459.3
Sugar		9⅓	1,029.4
Herbs		3⅓	.....
Salt	.....		.....
Total calories from institutional diet	.....		521,014.58
Calories from institutional diet per day per child	.....		930.38
Milk calories per child per day	.....		580.24
Calories per child per day	.....		1,510.62

TABLE XLVIII—*Continued*

(b) INSTITUTIONAL DIET

[Diet for two weeks; 92 children]

Food	*Weight*		*Calories*
	Lbs.	*Oz.*	
Hominy		8	804.5
Rice	1	0	1,591.0
Barley	12	9	20,225.4
Flour	2	8	4,007.6
Oatmeal	69	8	125,307.8
Bread	1,312	0	1,540,288.0
Green corn		14	401.8
Dried beans	1	0	1,564.0
Fish	10	0	13,920.0
Beef	60	14	43,160.2
Potatoes	12	11	4,795.6
String beans	18	10	3,520.0
Carrots	2	14	389.2
Onions	3	14	853.2
Turnips	1	14	335.8
Cabbage	12	9	1,796.1
Eggplant	5	11	739.1
Squash		12	160.8
Preserves	24	0	38,040.0
Condensed milk		18⅔	1,792.0
Drippings		3	639.3
Sugar		14	1,544.2
Total	.....		1,806,075.6
Calories per day per child	.....		1,402.23

TABLE XLIX

(a) Milk Group

[Diet for one week; 39 children]

Food	*Weight*		*Calories*
	Lbs.	*Oz.*	
Hominy	3	0	4,824.0
Rice	6	2	9,744.8
Barley	2	12	4,427.2
Flour		12	1,177.2
Macaroni		8	812.0
Oatmeal	11	0	19,833.0
Cornstarch	1	0	1,632.0
Corn meal	8	14	14,315.2
Bread	136	14	160,691.6
Dried peas		12	1,208.4
Liver		8	269.6
Pig tail		4	724.8
Veal	2	15	1,654.0
Beef	12	1	8,552.3
Potatoes	5	6	2,031.6
Carrots	3	1	627.8
Rutabaga		15	177.0
Turnips	5	14	1,051.8
Parsnips	4	2	1,237.4
Onions	2	0	440.0
Celery	1	10	136.0
Cabbage	6	4	893.6
Smoked cod	2	4	812.4
Sweet potatoes	3	12	2,092.8
Preserves	6	0	9,510.0
Bananas	4	14	2,178.6
Condensed milk		9⅓	896.0
Whole milk	595	0	.....
Fat	1	4	4,262.4
Sugar		5⅓	588.2
Salt		3	.....
Parsley	.....		.....
Total calories from institutional diet	.....		256,801.7
Calories from institutional diet per day per child	.....		940.6
Milk calories per child per day	.....		580.24
Calories per child per day	.....		1,520.84

TABLE XLIX—*Continued*

(b) INSTITUTIONAL DIET

[Diet for one week; 93 children]

Food	*Weight*		*Calories*
	Lbs.	*Oz.*	
Hominy	10	0	16,080.0
Rice	2	0	3,182.0
Barley	5	0	8,050.0
Macaroni	1	2	1,827.0
Oatmeal	16	8	29,748.8
Corn meal	14	0	22,582.0
Dried peas	1	8	589,641.6
Bread	502	4	2,417.6
Lima beans	2	8	3,964.8
Kidney		9	183.6
Liver	4	10	2,489.0
Ear (as tripe)		10	163.0
Heart	3	13	5,032.5
Beef	36	0	22,524.0
Bologna		12	850.8
Bacon		12	1,947.6
Wieners	5	0	5,760.0
Corn beef	7	2	9,055.8
Potatoes	5	6	2,031.6
Dried beans	1	8	2,345.6
Dried lima beans	2	8	3,964.8
Carrots	4	0	820.0
Turnips	16	1	2,875.2
Salsify	1	11	.....
Parsnips	14	13	4,443.1
Onions	3	15	867.0
Cabbage	2	11	383.9
Celery		10	52.0
Eggplant		12	97.2
Salt mackerel	15	0	20,880.0
Sweet potatoes	17	14	9,974.6
Preserves	12	0	19,020.0
Bananas	11	10	5,196.0
Apples	6	8	1,391.2
Condensed milk	12	13	19,680.0
Fat		½	106.5
Sugar		7	793.8
Salt		0	.....
Parsley		3	.....
Total	.....		820,422.6
Calories per day per child from institutional diet	.....		1,260.2
Supplementary feedings:			
Sirup	.....		17,466.66
Sugar	.....		4,626.72
Bread	.....		38,021.2
Total	.....		60,114.58
Total calories per day per child	.....		1,492.3

to pair them so that any child in one group was quite comparable in size, age and condition to one in the other.

It is to be expected in any demonstration of this kind, where tuberculous children, and children who had suffered from faulty skeletal development to a pronounced degree, were included, that certain individuals would be less capable of responding to an improved dietary with growth than would others. The accompanying charts show the general results of the comparison.

One striking feature of the records of both groups is the sharp increase in body weight, and at a more rapid rate than normal, in the first four to six weeks they were under observation. This happened to both the milk-fed and check group. We cannot determine with certainty the cause of this sharp increase in weight in both groups, but the most plausible explanation is that our observation of the institution led to an increase in the quantity of food provided to all children of the institution just at this time. There is reason to believe that before our advent the children were being fed a diet which was deficient not only in quality but also in amount.

In the check group, where the quality of the food remained essentially constant before and during the demonstration, the sharp increase in weight of 2 to 5 pounds was not continued. The weight curves of this group suddenly flattened, and in practically all cases remained nearly or quite stationary. In several children there was a gradual loss in weight during the first 12 to 15 months of the demonstration. Only one child in the check group made any appreciable gain during the first 15 months.

The milk-fed group stood in marked contrast with the check group in several respects. The sharp rise in body weight which was observed in both groups during the first few weeks was continued in most children throughout the demonstration, which for a considerable number covered a period of 21 months. One child of five years, who weighed but 28 pounds in the beginning, increased its body weight by 70 per cent. at the end of 8 months on the milk, 90 per cent. at the end of the first year, and reached a weight somewhat over the normal curve as given by the Holt, Boas and Burke table. Several increased 50 per cent. or more in weight during the first year.

There was a marked change in the behavior of the milk-fed group as contrasted with the check group. The latter were apathetic and very tractable. The discipline of the institution was strict and these children were all obedient. Those in the

milk-fed group, on the other hand, soon caused annoyance to their teachers by their restlessness and desire for activity, and were frequently guilty of infractions of the rules.

The results of supplementing the institutional diet with milk were so striking that after 15 months it was decided to supplement the diet of the check group with a quart of reconstituted milk per day, in order to demonstrate whether any of these children possessed the capacity to grow which they were not manifesting on the institutional diet. During the next 6 months a considerable number of the check group grew at rates comparable to what had been observed in the original milk group. All things considered, the results of this demonstration constituted, we believe, a demonstration of the validity of the view that a dietary selected from cereals, tubers, fleshy roots and meat does not prove satisfactory for the physical development of young children. It shows further that milk is an effective supplementary food for such a type of diet; this has been repeatedly shown to be the case with animals.

In making these tables we selected three periods (two of two weeks each and one of one week) and gave the diet for the milk-group and the diet of one of the check groups only. In the last set of tables we selected a period when supplementary feeding (sirup, sugar and bread) was being given to the check group.

Very few of the children were able to gain in weight on the institutional diet. Many who were not able to do so responded with growth at rates greater than the normal expectation when the institutional diet was supplemented with a quart of milk per child per day. In a number of cases children grew well while receiving the milk, but declined in weight when after several months they were returned to the institutional diet. Several children who were unable to gain on the institutional diet grew at phenomenal rates with the milk supplement. There was no evidence in the records of any seasonal variation in the rate of growth.

On the whole, these data show that there was much difference in the vitality of different children. Some were able to grow on a diet which was obviously too poor to induce any growth in others who were less well endowed physically. Such a difference we have often seen in young animals. The results emphasize the fallacy of assuming that because a somewhat poorly selected dietary induces apparently normal development in one child, is it good enough for all children. The aim should be to

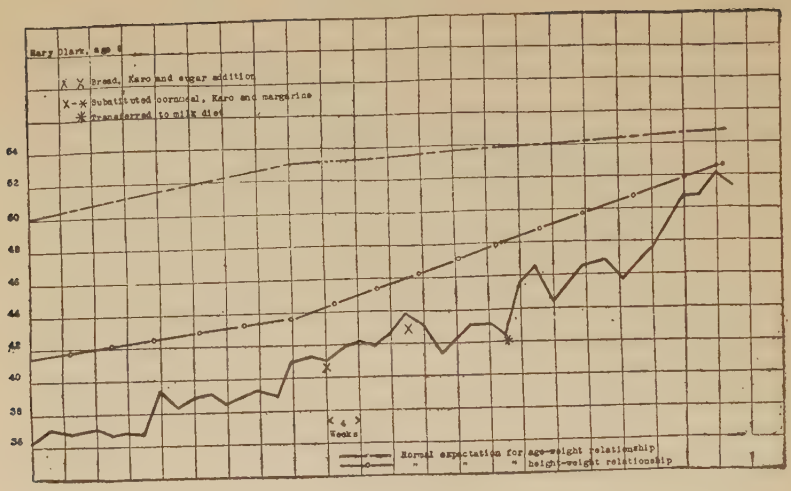
plan the diet of the child so as to make it as nearly perfect as possible.

Observations on American School Children.—The children in this institution represented a somewhat exaggerated picture of what exists almost everywhere throughout this country among children in private homes. The results of our study, supplementing as they do the extensive data of animal experimentation, fully justify the concern of those who have been active within recent years in making surveys of the physical condition of school and pre-school children in cities and in country districts. Improvement of the dietary is of the greatest significance for the welfare of a large percentage of American children of the present generation. Leete (1921) summarized the statistics of examination of a large part of the twenty million school children in the United States. She found one per cent. to be mentally defective, 5 per cent. were or had been tuberculous, 25 per cent. had defective vision, 15 to 25 per cent. had diseased tonsils or adenoids, 10 to 20 per cent. had deformed feet, 50 to 75 per cent. had defective teeth, and 15 to 25 per cent. were suffering from malnutrition.

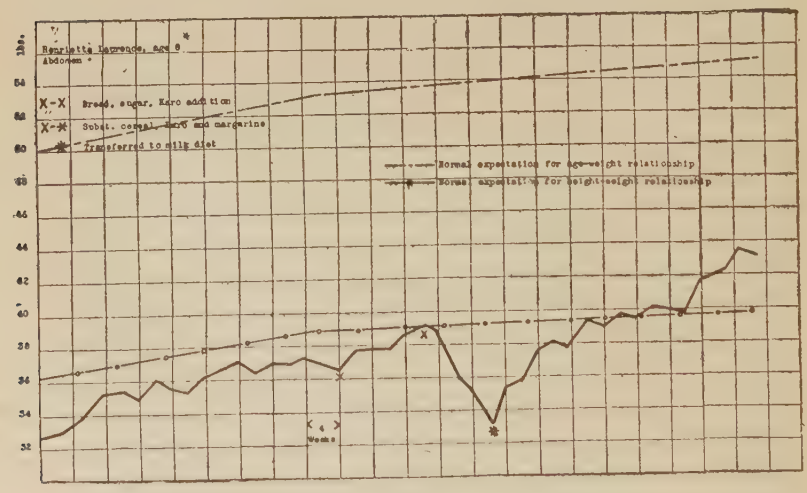
Terman (1914) stated that about fourteen million of the twenty million school children in the United States were handicapped by some kind of physical defect, and that not far from two million were suffering from a grave form of malnutrition. Ten million are said to have enough defective teeth to seriously interfere with health. That the conditions are fully as bad as these figures indicate was borne out by the results of medical examination of young men for the draft in the World War.

Supplementary Feeding of School Children.—Morgan and co-workers (1926, 1927) have made several studies on the effect of small supplementary feeding on the growth of school children. The addition of small amounts of figs, oranges, or milk result in a marked increase in weight and general well-being. Their papers should be consulted for details. Morgan and Warren (1928) have continued these studies. They studied 177 children in attendance at a public school in Oakland, California, to determine the stimulation of growth induced by small supplementary feedings. Four groups were formed, one of which did not receive extra feeding. The others received half a pint of milk, one orange, and a small cracker sandwich, respectively, each school day for ten weeks. The children in all three groups given supplementary feedings made large gains in growth as compared with

Original check group.

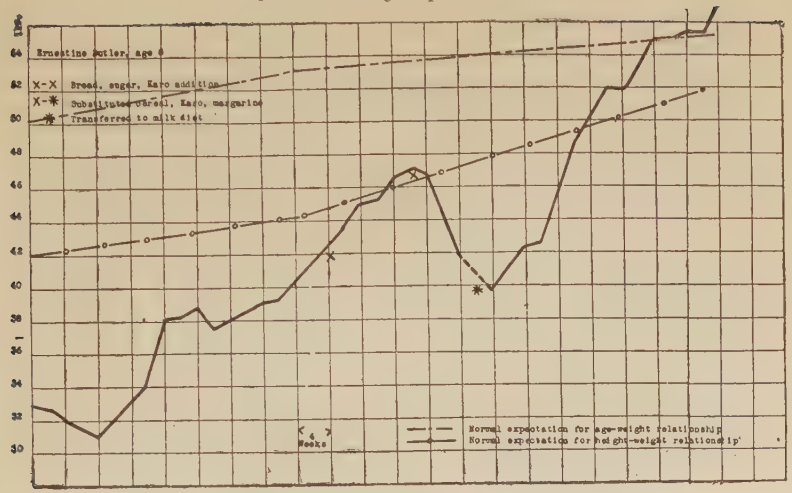


a

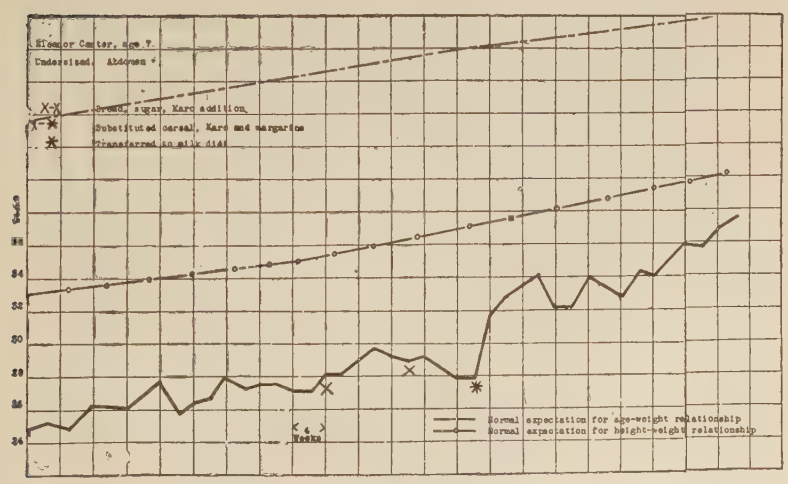

Henrietta Lawrence, age 8
Abdomen *
X-X Bread, sugar, Karo addition
X-* Subst. cornmeal, Karo and margarine
* Transferred to milk diet
Normal expectation for age-weight relationship
Normal expectation for height-weight relationship
4 weeks

b

CHART XIII—*a-b*. Original check group.—Received institutional diet plus supplementary feeding (for calories), but gained little or nothing until milk was added, when they grew. Upper broken line, normal expectation for age-weight relationship. Lower broken line, normal expectation for height-weight relationship. Solid line, growth curve. * Transferred to milk diet.



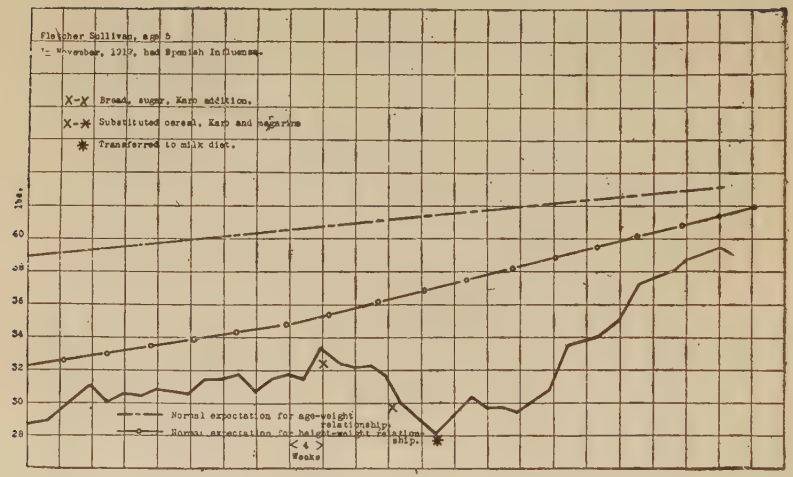
c



d

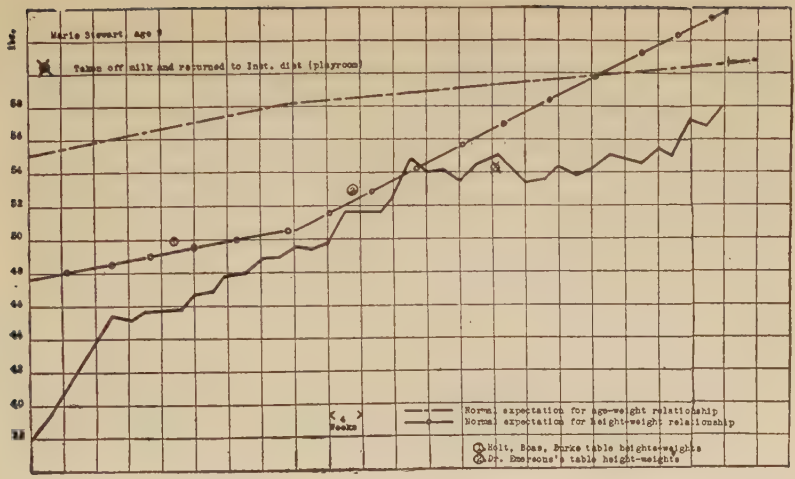
CHART XIV—*c-d*. Original check group.—Received institutional diet plus supplementary feeding (for calories), but gained little or nothing until milk was added, when they grew. Upper broken line, normal expectation for age-weight relationship. Lower broken line, normal expectation for height-weight relationship. Solid line, growth curve. * Transferred to milk diet.

Original check group—Continued.

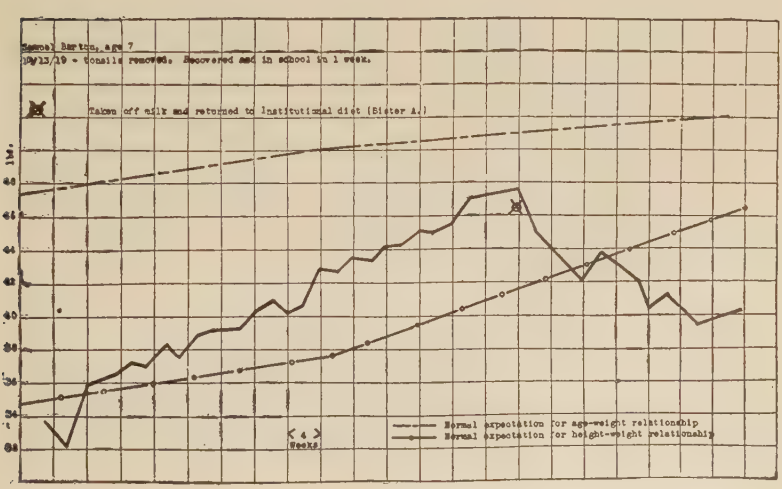


e

CHART XV—*e*. Original check group.—Received institutional diet plus supplementary feeding (for calories), but gained little or nothing until milk was added, when they grew. Upper broken line, normal expectation for age-weight relationship. Lower broken line, normal expectation for height-weight relationship. Solid line, growth curve. * Transferred to milk diet.

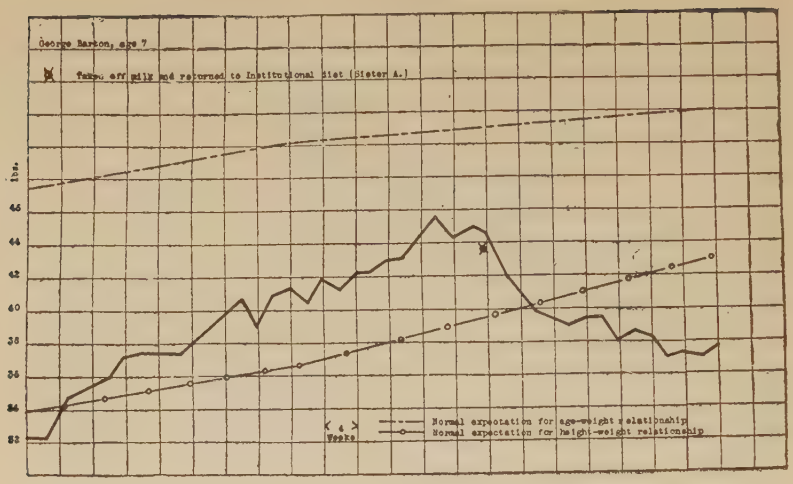

Marie Stewart. Age 9
Taken off milk and returned to Inst. diet (playroom)
Normal expectation for age-weight relationship
Normal expectation for height-weight relationship
Holt, Boas, Burke table heights-weights
Dr. Emerson's table height-weights
4 Weeks

a

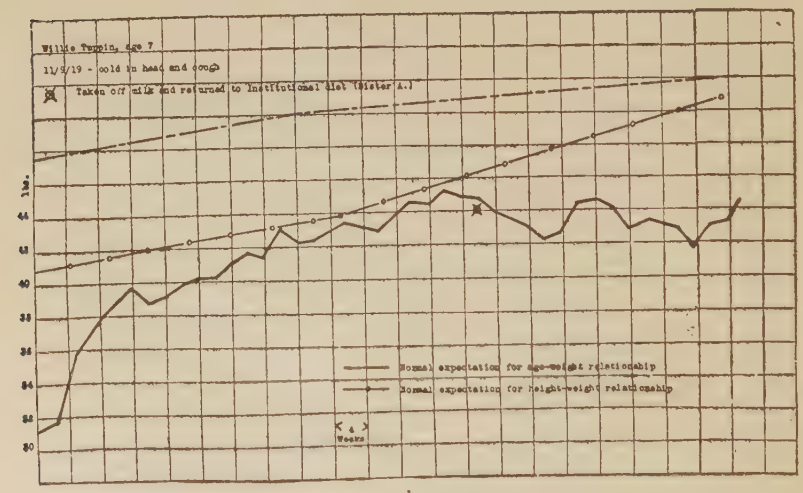

Taken off milk and returned to Institutional diet (Dieter A.)
Normal expectation for age-weight relationship
Normal expectation for height-weight relationship
4 Weeks

b

CHART XVI—*a-b*. Original milk group.—On milk diet for some months. When taken off milk diet and put on institutional diet, either lost weight, failed to gain, or gained but little. Upper broken line, normal expectation for age-weight relationship. Lower broken line, normal expectation for height-weight relationship. Solid line, growth curve. * Taken off milk diet.



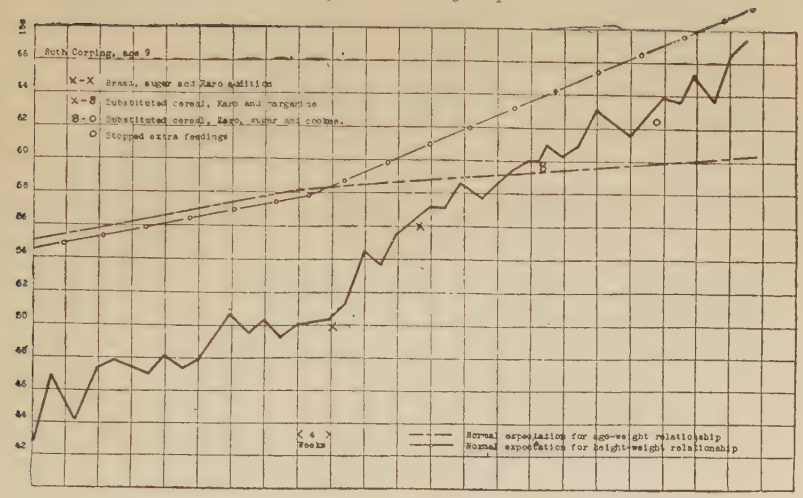
c



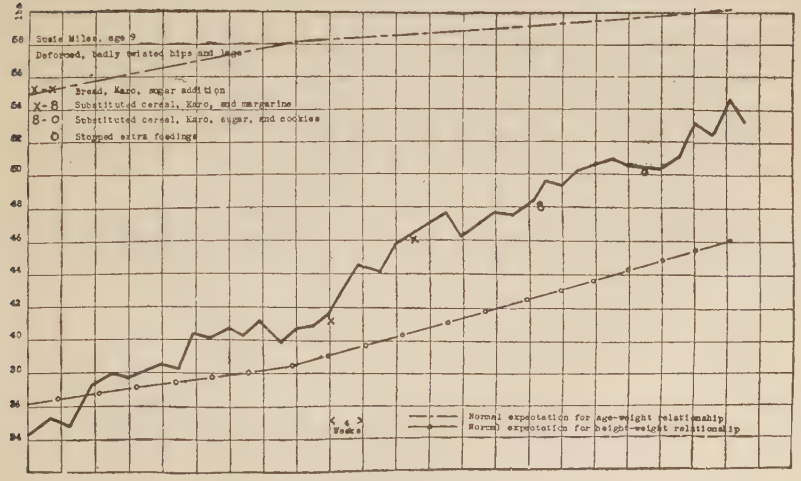
d

CHART XVII—*c-d*. Original milk group.—On milk diet for 13½ months. When taken off milk diet and put on institutional diet, either lost weight, failed to gain, or gained but little. Upper broken line, normal expectation for age-weight relationship. Lower broken line, normal expectation for height-weight relationship. Solid line, growth curve. * Taken off milk diet.

Original check group.


Ruth Corring, age 9
X-X Bread, sugar and Karo addition
X-8 Substituted cereal, Karo and margarine
8-O Substituted cereal, Karo, sugar and cookies.
O Stopped extra feedings
Weeks
Normal expectation for age-weight relationship
Normal expectation for height-weight relationship

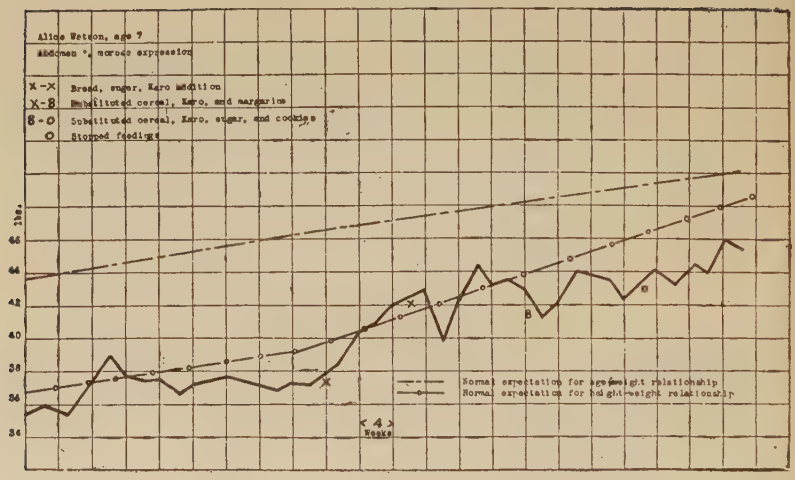
a


Susie Miles, age 9
Deformed, badly twisted hips and legs
X-X Bread, Karo, sugar addition
X-8 Substituted cereal, Karo, and margarine
8-O Substituted cereal, Karo, sugar, and cookies
O Stopped extra feedings
Weeks
Normal expectation for age-weight relationship
Normal expectation for height-weight relationship

b

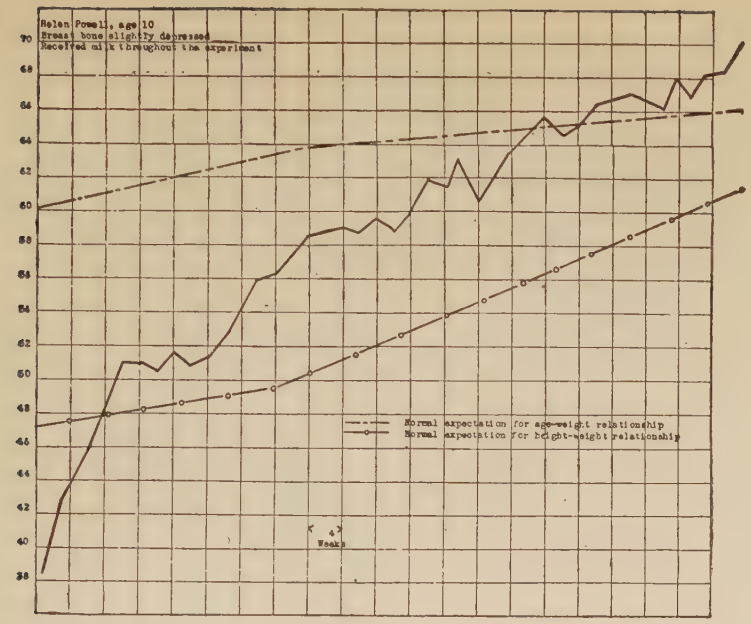
CHART XVIII—*a-b*. Original check group.—Received institutional diet plus supplementary feeding, and gained. Upper broken line, normal expectation for age-weight relationship. Lower broken line, normal expectation for height-weight relationship Solid line, growth curve.

Original check group—Continued.

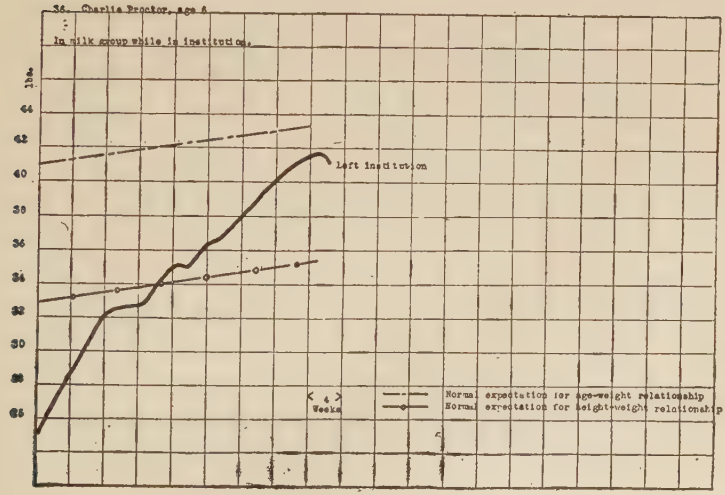


c

CHART XIX—*c*. Original check group.—Received institutional diet plus supplementary feeding, and gained. Upper broken line, normal expectation for age-weight relationship. Lower broken line, normal expectation for height-weight relationship. Solid line, growth curve.

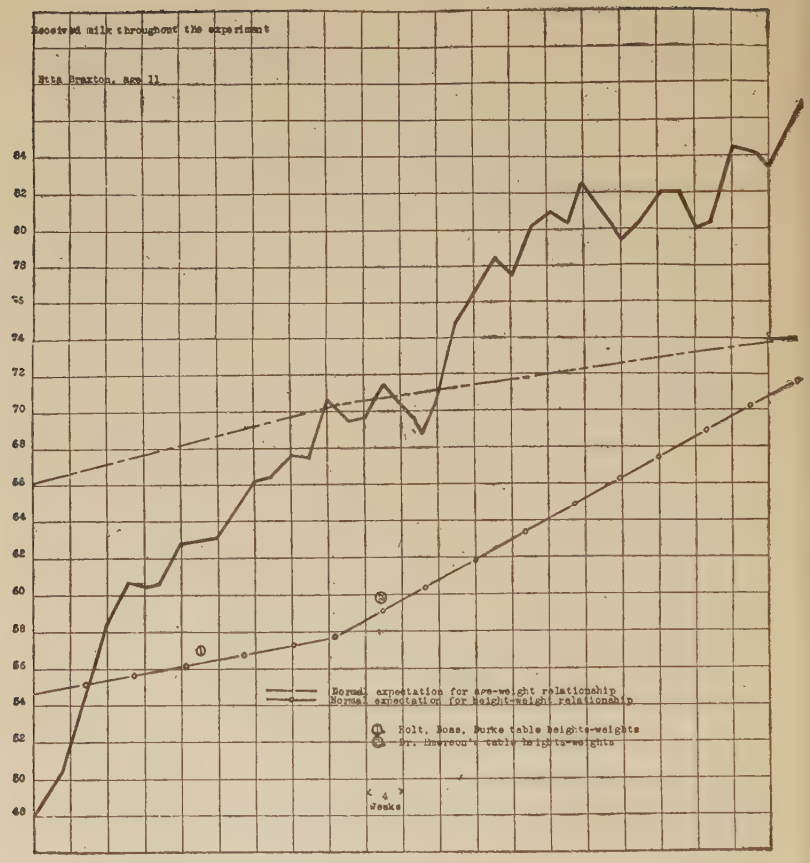


a



b

CHART XX—*a-b*. Original milk group.—Received milk throughout experiment and gained remarkably. Upper broken line, normal expectation for age-weight relationship. Lower broken line, normal expectation for height-weight relationship. Solid line, growth curve.



c

CHART XXI—*c*. Original milk group.—Received milk throughout experiment and gained remarkably. Upper broken line, normal expectation for age-weight relationship. Lower broken line, normal expectation for height-weight relationship. Solid line, growth curve.

the controls. The group fed on milk and crackers gained weight at about the same rate, and the group fed on oranges somewhat less. The group fed on oranges, however, made better gains in height than the others. The mere addition of extra calories furnished a measurable advantage for these children.

Diet and Span of Life.—The extreme view has often been expressed that heredity and external environment are the only significant factors in determining the length of life. Unfortunately the environmental conditions of a life-time in man cannot be ascertained with much definiteness, and the dietary habits of people are so variable from time to time that hitherto they have not afforded any basis of discussion in relation to longevity. McCollum and Simmonds have repeatedly emphasized an interrelationship between the quality of the food supply and the span of life of experimental rats, and have pointed out that with our present knowledge it is easily possible to make the span of life of this species anything that is desired up to perhaps 40 months. Recently Sherman and Campbell (1928) made a study, using the rat, of the effects of two diets consisting of wheat and milk, both nutritionally adequate but differing in the proportions of the two components. They used 400 rats of the same heredity and living under the same conditions. The animals receiving the higher proportion of milk lived on an average 10 per cent. longer than did the other group. From the statistical point of view it is stated that there was not more than one chance in 100 that this result could have been accidental due to natural physiological variability of the animals. The superior longevity induced by improvement of the already adequate diet was further shown in that much larger percentages of both sexes attained definite standards of longevity on the improved than on the original dietary.

Family Deterioration Due to Sub-optimum Diet.—The results of observing animals beyond the completion of growth to determine their fertility, success in rearing young, and the rate at which they developed the characteristics of old age, were so striking that we early extended our observations to include restricting single families to a monotonous diet through several generations. This brought to light the fact that a diet may be sufficiently good to enable an animal to grow in a perfectly normal manner, and to exhibit a moderate amount of fertility, yet with each succeeding generation the progeny became smaller and less well developed physically, and after two, three or four gen-

erations the strain died out. Several comparable examples of similar experience in man may be cited. Campbell (1889) stated that among the poorer classes in the slums of English cities the family never passes beyond the third generation. Galton (1906) in a statistical analysis of one thousand town families and one thousand country families selected from the town of Coventry and the surrounding rural population, found that the town population supplied to the succeeding generation but three-quarters of the number supplied by a similar number of country people. In two generations the adult grandchildren of the town dwellers amounted to but little more than half the number of descendants of the country dwellers. The employed urban dwellers are continually being recruited from country stock, and themselves tend to die out. Food is cheaper in rural districts and the selection and quality is distinctly better, generally speaking, than it is where the supply is derived almost entirely from the grocery store. The grocer carries a surprising proportion of stock derived from cereal grains, legume seeds, fruits, tubers and muscle meats; it has been emphasized that food-stuffs from this list cannot be combined to constitute a satisfactory diet. The behavior of our rat population when restricted to diets corresponding in their origin to those of the urban dweller of either England or the United States is illuminating. The extent to which the rat has proven to be sensitive to even small modifications in its diet is truly amazing. These observations suggest that it is much more important than hitherto believed to have the diet nicely adjusted with respect to all its essential constituents, in order that development shall proceed uninterruptedly and the health of the individual be maintained as near the optimum as possible. It is under such conditions that the characteristics of youth are preserved as long as possible and the span of life prolonged.

Climate as a Factor Influencing Nutrition.—Huntington (1915) has discussed climate as being one of the most important factors in determining the development of a highly progressive race or nation. His studies show that wherever the climate has a favorable range of temperature change from day to night, and is characterized by storminess of the cyclonic type, the people show energy and aggressiveness. As a result of his studies he concludes that a mean temperature of 64° F., a mean humidity of about 80 per cent., and frequent changes of temperature are the most desirable conditions for physical work. Statistical evi-

dence is offered to support the theory that only people who live under conditions of relative storminess become industrious, capable and successful.

Japan in respect to storminess ranks as one of the most stimulating regions of the world. It was pointed out in Chapter XXVII that the children born of Japanese parents in California were larger and better developed physically than the children born in Japan. It seems almost certain that a dietary factor is operating to produce these results.

Achievements of Peoples of Canada and of the Bahamas.—Although climate is undoubtedly a factor of great importance in determining well-being and consequent achievement, geographers have ignored facts which modern research has brought to light in the field of nutrition, which should find a place in any discussion of human progress. A contrast made by Huntington is that of the virility of the Canadians and the improvidence and laziness of the Bahamans. He points out that at the time of the American Revolution certain loyalists in the northern states preferred to emigrate to Canada rather than to live under the Stars and Stripes. Other loyalists in the south betook themselves to the inviting climate of the Bahamas for a like reason. It is agreed that their ancestors were equally capable and aggressive, although of very different mental types. The descendants of these Canadians at the present time are industrious, educated and progressive. Those of the Bahamans are indolent and unprogressive, and have suffered retrogression since their settlement in the islands. The explanation offered for this difference in quality in the two groups to-day is that the climate of lower Canada is a highly stimulating one, whereas that of the Bahamas is too mild and uniform. It is not permissible to attribute the differences observed between these two peoples solely to climate, for their diet has been so different that there is good reason to suspect that the attitude toward work and mental effort in the two groups is in no small measure due to the way they feel. The Bahamas produce tamarinds, oranges, lemons, olives, limes, citrons, pineapples, pomegranates, figs, sapodillas, bananas, melons, yams, potatoes, cucumbers, cassiva, sugar, maize, and peas as the chief articles of food. The breadstuffs and meat are imported from the United States. According to animal experiments this list of foods would not form a satisfactory diet. The people of lower Canada have taken a diet which contained rather liberal amounts of milk, butter, eggs, cream, and cheese, in addi-

tion to cereal, legume seeds, tubers, and meats. People will feel very different on these two types of diets.

Stimulating Climates not Promoting Health in Man.—If climate were responsible for health to the degree to which geographers believe, how are we to account for the amount of sickness, early aging and physical inefficiency seen in the most favorable climatic areas such as the northeastern quarter of the United States and England, and the fine physical development of the Arabs and the pastoral tribes of Asia, whose climate is less stimulating? The primitive peoples of Fiji, Hawaii, and Samoa were of exceptional physical perfection in a uniform and mild climate. It has been pointed out that the teeth of the Americans and English of the present generation are indescribably bad, even where the climate is said to be best adapted to health and to the promotion of physical and mental activity. What stands in the way of the progress of Asiatic and African peoples which has kept them far behind the Europeans and Americans in advancing learning? It seems plausible to regard the failure of certain peoples to progress as primarily a geographic one quite as much as climatic. The nature of the land and the amount of rainfall determine the character and vigor of its vegetation, and indirectly the character of the diet. Climate determines the kind of agriculture which is profitable and therefore the most satisfactory mode of life of the people of any region. Hookworm, malaria, etc., complicate the situation.

Health of American Pioneers.—Poor physical condition is by no means restricted to recent years in this country, nor is it necessarily due to modern conditions of city living. Many will recall how our grandmothers insisted upon the frequent treatment with sulphur and molasses, the periodical dosing with bitters, and the efficacy of sassafras tea in the spring as a means of "thinning the blood," which was supposed to have become "impure" and "thick" during the winter. Among our pioneer ancestors the idea was generally accepted that there was need for a "spring medicine" of some kind. This belief rested on common experience. In the early settlement of many of the states the people suffered great hardships. With little capital and no food reserve their winter diet was generally very simple and monotonous. There is good reason to believe that it was chemically unsatisfactory for the maintenance of health. After a period of several months during each succeeding winter they felt "run down." The almanacs, which were circulated by patent medi-

cine concerns, discussed the prevalence of "that tired feeling" in the spring. They convinced people that they needed a course of treatment with sarsaparilla or a "spring tonic" or other "blood purifier." The use of these tonics was so universal that it established the belief that winter was an unhealthful season.

If one now investigates the same communities where a few years ago this condition was regularly observed, one finds well equipped farms, supplied with dairy herds, orchards and all the signs of modern prosperity. There is much less difference between the winter and summer diet, due to the abundance of canned fruits and vegetables put up at home or procurable from the stores. More cabbage, turnips, apples, celery, etc., are regularly provided for the winter season. These changes have caused the disappearance of the "tired feeling" in the spring, or better, at the end of winter, and consequently the need for "spring medicines."

The vendor of "spring medicines" had a powerful ally in the spring diet of his customers. With the coming of warm weather various "greens" were available and were eagerly consumed. Clover sorrel pies, wild onions and dandelions grew in abundance on the prairies and were added promptly to the food supply. The cows, which had been dry all winter due to their starved condition, became fresh, and milk was available. The few hens in the barnyard added worms, insects and tender grass to their diet and began to lay eggs. The garden furnished an abundance of fresh vegetables which were eaten with a relish which can be appreciated only by one who has for a period been semi-starved. The "tired feeling" disappeared about this time, but the regular spring remedies had been taken and the beneficial results of better diet were wrongly attributed to the efficacy of drugs.

Ehrstrom of Finland (1924) in discussing "spring tiredness" and vitamin deficiency, states, "We are well in the autumn after the light and open air in the summer and we feel unwell in the spring after the darkness of the winter." Possibly the absence of sufficient vitamin C in the winter diet in Finland may have contributed to deterioration of health in winter.

Importance of Properly Constituted Therapeutic Diets.—Davidson (1928) has recently called attention to three cases in which manifestations of deficiency diseases developed as the result of the physician directing his attention to the dietotherapy of an obvious pathological condition to a degree which resulted in ignoring the general laws of nutrition. One of these patients

suffered from pernicious vomiting, another from vomiting and hematemesis believed to indicate gastric ulcer, and a third from gastric ulcer. In these three patients with digestive disease during the course of an ulcer diet regimen, manifestations of deficiency diseases developed, as evidenced by dental caries, bleeding, spongy gums, purpura, and intra-muscular hematomas. The necessity of adequate accessory food factors in special diets, as prophylactic against the development of deficiency diseases, and the ensuing possible detrimental effects on the disease under treatment, must be emphasized. Shattuck (1928) points out that the polyneuritis seen in this country in various debilitating diseases such as tuberculosis, cancer, diabetes, syphilis, chronic alcoholism, or the marasmus of infants, may possibly be caused by a deficiency of vitamin B in the diet or to a failure to assimilate food carrying this factor. He thinks some cases of polyneuritis may possibly be properly regarded as true beri-beri and not as complications or sequelae of the other diseases.

From many sources it appears that insufficient attention is directed to the application of the principles of our newer knowledge of nutrition to the diet in disease. In view of recent advances in nutrition there is no doubt that much could be done to aid the patient in the battle to regain his health if more attention were given to the details of his diet. It is not within the scope of this book to give a detailed presentation of the application of principles to the dietary management of various diseases.

The Most Satisfactory Type of Diet.—The system of diet which can be recommended with assurance that it will go a long way toward improving the physical fitness of the nation is a very simple one, and its daily practice involves no great self-denial. It involves the borrowing of the best elements from those several systems of diet which have been thoroughly tested in human experience and found successful.

The first and most important principle is the extension of the use of dairy products.* Instead of the present consumption of half a pint of milk a day, there should be about a quart per capita. This is a feature of the diet of all the pastoral peoples

* It would be beyond the scope of the present discussion of nutrition to present in an adequate way the details of putting into effect in the home the ideal system of diet. We have treated this subject elsewhere from the point of view of the housewife. (*Food, Nutrition and Health*, 2nd ed., 1928.)

of the past and present which has made them superior in physical perfection.

The second principle is that there are dietary properties in the leafy vegetables which are unique among foods of vegetable origin. These have been the "protective foods" for many of the Asiatic peoples. Their regular consumption in liberal amounts serves not only to provide valuable nutrients not contained in adequate amounts from milled cereals, tubers and muscle meats, but also to maintain the intestinal tract in a hygienic condition through promoting elimination. Milk likewise serves, through its encouragement of the growth of lactic acid-producing organisms (which cause souring of milk), to bring about the disappearance from the intestine of those types of bacteria which cause putrefactive decomposition of the food residues with the production of substances which are a physiological abomination. This principle, first emphasized by Metchnikoff, has been shown by bacteriological methods to be sound. Milk has dietary properties which the famous bacteriologist did not discern, and which make it the one food for which there is no effective substitute.

It is essential to keep in mind a third principle of great importance in nutrition, viz., that of taking daily a certain amount of raw vegetable food to provide a sufficient amount of the antiscorbutic substance. If these simple principles are adhered to, the main features of an adequate diet will be fulfilled, and the remainder of the food supply may safely be derived from any of our ordinary milled cereal products, tubers, root vegetables, sugar, and meats. Infants and children can be safeguarded in their skeletal development by providing at regular intervals a suitable amount of cod liver oil, and by affording an opportunity for outdoor exercise in sunlight.

Since the foundations of physical well-being are laid in prenatal life and early infancy, the importance of an adequate diet for the expectant and nursing mother cannot be too strongly emphasized. No gift we may bestow upon our children can compare with the priceless endowment of physical integrity without which they cannot achieve the maximum of health which is their just heritage. Society must measure its well-being in terms of the health of its children.

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